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SEED DEVELOPMENT IN *MEDICAGO* (ALFALFA) HYBRIDS

I. THE NORMAL OVULE[1]

BY HELEN M. FARLEY[2] AND A. H. HUTCHINSON[3]

Abstract

Seed production in *Medicago* (alfalfa) hybrids is related to many factors, primarily the cytological disturbances due to hybridization. The establishment of new balances results in strains which may occupy different habitats. Deficiencies may appear at one or more of the many stages during the complete process of growth and development. This account on the normal ovule, as a standard for comparison, deals particularly with the histogenesis and development of the nucellus, gynospores, integuments, funiculus, female gametophyte, embryo and endosperm. Some of the food and nutrition problems associated with seed production are considered. A series of papers dealing with special phases of seed development in hybrid *Medicago* is in progress.

Introduction

Cultivated forms of alfalfa are notably low seed producers. Previous descriptions of the histological development of the ovule and seed in alfalfa have applied to plants designated as *Medicago sativa* L., presumably a pure species; since the plants described in this paper and most of the northern, commercial forms of alfalfa are of hybrid origin, it has seemed to the authors to be significant to determine the degree of variation from the "original species" as well as the variation within a hybrid group.

Previous descriptions of ovule development in seed plants have given much emphasis to the direct precursors of the egg and embryo, that is to the female gametophyte, and minimal attention has pertained to the adjacent protective and nutritive tissues. Recent investigation, however, has emphasized the importance of the nutrition balance after embryo formation. The dependence of egg production in plants upon favourable nutritive and protective structures has been taken for granted and the uniform accomplishment of this teleological necessity in development has been assumed, to a high degree. This assumption seems quite contrary to fact, at least in the hybrids of alfalfa examined in the present study. The greater part of this paper deals with the origin and development of nutritive and protective tissues leading to the production of normal eggs, as essential features of seed formation, especially in alfalfa hybrids grown at the University of British Columbia.

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Material

Early History of Medicago

The earliest history of man records the fodder plant, now known as *Medicago*, in Media; probably the species was similar to *M. sativa* L. of the present and these plants were the ancestors of the several, recent, cultivated species and numerous varieties which are widespread, and of great economic importance. Later, it was grown in Persia and was carried to Greece by Alexander the Great; then, to Italy. The Moors are credited with its introduction to Spain from Africa, during the thirteenth century; they called it "alfalfa" or "best of fodder". During the sixteenth century the Spanish introduced it into Mexico and later to South America; from Chile the migrants of the gold rush carried the seeds to California. "By 1850 it had spread eastward to Utah, Colorado, Kansas, Nebraska, Iowa, Illinois, and Ohio" (25). This chain of translocations did not materially change the climatic habitat from its original "Mediterranean" type and the species would appear to have retained the primary characteristics of the ancestor from Media (14).

From Spain "alfalfa" was carried to southern France, and to Geneva during the sixteenth century (14). It seems probable that the term "lucerne" was applied first either to the form (known as "laouzerdo") grown in Southern France (17) or to a Swiss variant of *M. sativa* that was tolerant to mid-temperate conditions (14). German lowland lucerne was first (1710) developed by Cistercian monks in Franconia; later (1730) the evidence indicates that an upland form, sometimes called "*M. media* (Pers.)", and commonly called "lucerne" was selected from *hybrids** of the Franconian *M. sativa* L. × *M. falcata* (L.). The latter ancestor is believed to have originated in the Caucasus region, an area that is very rich in natural species of *Medicago* at present (11, 24).

"The first recorded attempt to grow alfalfa in the United States of America was made in Georgia in 1736 (25). These early attempts to grow the crop in the East were probably on acid soils which in part explains the unsatisfactory results" (Westover, personal correspondence, 1941). Although occasional fields of "lucerne" were grown on "limestone soil" as early as 1794 (23), the first *Medicago* to be more widely successful in the northern United States and in Canada was derived by natural selection from offspring of the German hybrid "*M. media*" and was cultivated on a Minnesota farm by Wendelin Grimm after whom it was named "Grimm". A similar form was selected in Canada from hybrid plants grown from seed of German origin, and was designated "Ontario Variegated". *M. sativa* is purple-flowered, *M. falcata* yellow-flowered and the hybrids vary in this as well as in other respects.

Origin of British Columbia Hybrids

The British Columbia hybrids are the result of a back-cross between plants of "*M. media*", variety "Ontario Variegated", and a single plant of *M. falcata*

**The problem arising from the difference between a Mendelian hybrid and a hybrid as defined by the International Rules of Botanical nomenclature is recognized by using italics where the word hybrid is used in a taxonomic sense.*

var. Don, obtained by Dr. L. S. Klinck from Prof. N. E. Hansen of South Dakota, who formerly imported this variety from Russia. The crosses, made by P. Boving, resulted in six back-cross hybrid plants with marked variation in gene combinations, notably in flower colour, pod shape and size, degree of seed production, extent of rhizome formation, and erectness of habit. Plants have been selected by G. G. Moe, from three generations of hybrids, with particular reference to the economic features: extent of rhizome production, erectness, and seed production; the improvement of these "hybrids" is progressing.

If the reported ancestry is authentic the '*falcata*' lineage was twice introduced in the production of the British Columbia hybrids, once at the origin of the upland German '*media*' and again (1916) from the 'Don' used in the British Columbia experiments. Through '*media*' the remote ancestry '*sativa*' enters, and it is possible that the development of 'Ontario Variegated' may have added another '*sativa*' factor to the sum total of this hybridity.

In this account, since the original British Columbia plants were of multi-hybrid origin they will be designated as *M. media* (B.C.) and *M. falcata* (B.C.), respectively, and their hybrids as *M. media–falcata* (B.C.) hybrids, Generations 1, 2, and 3, respectively.

Methods

Various killing and fixing reagents were used, especially Flemming's, La Cour's, Navaschin's, Bouin's, and Carnoy's. The most satisfactory results were obtained with combinations of alcohol, acetic acid, formalin, and chloroform. Tissues were hardened by osmic and chromic acids and by mercuric chloride.

No one strain was entirely reliable for all tissues. Iron alum haematoxylin (with light green counter stain), safranin with light green, Feulgen's carmine stain, and Semmens' and Bhaduri's light green counterstain were all utilized to advantage for particular stages or tissues.

Records are available for the immediate ancestry of each plant from which flowers were collected, and for the commercial characteristics. Flowers were marked at the time of tripping and subsequent collections were recorded from this reference point. Stages before tripping were estimated, with the same reference point.

The Problem of Determining Normality of Seed Development in Hybrid *Medicago*

Medicago has, notably, a low seed production. Because of the multiplicity of species, strains, and hybrids growing under varying conditions of location and season the factors that contribute toward the non-development of ovules are necessarily many and varied in their relative values (5, 6). The following factors have been suggested. 1. The pollen may be non-viable. 2. If self-pollination occurs self-incompatibility may intervene (1, 9, 13). 3. The usual insect pollination is dependent upon the variable activity of bees under different conditions (19). 4. The rate of pollen tube growth may not parallel the

normal development of the ovule and fertilization may not be consummated (4, 12). 5. The food supply to the pollen tubes, to the integuments, to the nucellus, to the gametophytes, to the endosperm, and to the embryo may not be properly apportioned or timed, and normal development may not continue. 6. Finally, parasites may destroy the seed at some stage of development (21). Any deficiency in one of these chief events in the processes involved in seed production may be sufficient to inhibit the normal production of viable seed. The problem in any particular case is to determine the limiting factor or cumulative group of factors. In order to determine deficiencies a standard of the "normal" is required. Although many have noted the variants in *Medicago*, the complete series of stages in all parts of the ovule that are essential for the final development of viable seed has not been entirely evaluated. *The normality of development in Medicago is not to be judged on a basis of the greatest number of cases following a particular course, but preferably upon the harmonious succession and co-ordination of stages that attain the goal of seed production.* It is proposed to deal in later papers with the development of androspores and of the male gametophyte, polyploidy, and aneuploidy in relation to autosyndesis and compatibility, and the factors that lead to normality or to deficiencies in the processes of seed development.

The complex genesis of plants of "*M. falcata*" and "*M. media*" used as the progenitors of the British Columbia hybrids, added to the hybridization of the experimental B.C. *Medicago* lines, has resulted in a complex of variants, partly as a result of the Mendelian assortment and recombination of genotypic characters and partly as induced disturbances of the chromosome complements and their multilateral associations. These involve the physiological and genetic interrelations of the co-vivial generations included in the seed: the haploid gametophytes, male and female, the diploid sporangium, and the triploid endosperm. When polyploidy (20) ensues the balance is in further jeopardy. If new, successful, true-breeding strains are to be obtained, there must be established new equilibria that satisfy the requirements of growth vigour, of adjustment toward the particular environment, of intraracial compatibility, and of inter-racial incompatibility.

In the maze of variability, the recognition of a standard of normal development is the first problem of the geneticist; this standard may vary within certain limits of known divergence, limits beyond which deleterious results occur and are expressed as a marginal lowering of seed production or of seed viability.

Early Development of the Ovule, and Gynosporogenesis*

The single carpel of the *Medicago* flower develops from a terminal or subterminal, crescentic primordium on the floral axis and rapidly produces a distally open hemicylinder, with a suture along the infolded margins of the wall. Later, the margins fuse and the upper portion becomes constricted

**The terms "gynospore" and "androspore", as suggested by J. Doyle and by L. W. Sharp and as accepted by Thomson (22, p. 43), have been used in this account.*

to form the style; the somewhat inflated lower portion, the ovary, produces ovules near the margins of the infolded wall. When the elementary placenta is four cells in thickness the first differentiation of ovular meristem occurs; the dermatogen (outer tunica) actively divides anticlinally, extending the surface, and the meristematic margins of the ovary wall are thickened by coincident periclinal divisions of the hypodermis. On the inner side of the marginal fold the hypodermal layer (outer periblem or inner tunica) divides by regular periclinal divisions; on the outer side of the fold, at intervals along the margin, the ovular mounds develop. These discontinuous extrusions are the result of repeated periclinal divisions of subtending hypodermal cell groups, accompanied by a repression of divisions in adjacent cells (Fig. 3). Ordinarily a group of 10 or more hypodermal cells divide, that is three or four in sectional view, whereas occasionally a smaller number and possibly a single cell may be meristematically distinguished from the adjacent inactive hypodermal cells. This active cell, or cells, may be designated the potential *gynosporangial initial(s)*.

The carpel wall in the placental region is now five cells in thickness except at the ovular primordia which have a sixth and soon a seventh layer; the *primary hypodermal cells* have divided periclinally to produce *secondary hypodermal cells* and inner cells, which may be known as *basal* (tapetal) or *endoparietal* cells. Normally, a single secondary hypodermal cell, centrally located within a group, becomes larger, and denser, and differentiates into an *archesporial cell*. This, in turn, divides periclinally to produce the *gynosporocyte* and an *exoparietal cell*. Concurrently or shortly thereafter the endoparietal (basal) cell divides periclinally, giving an axial row consisting of four cells, a gynosporocyte, one exoparietal, and two endoparietal cells (Fig. 4). Not infrequently several rows develop similarly and several gynosporocytes are formed (Fig. 6). These may produce four gynospores each. Normally, when a single axial gynosporocyte differentiates, the adjacent, somewhat distorted rows of similar hypodermal origin, develop as nucellar tissue, lateral to the axial row (Figs. 3 to 8). Normal development here, as in the case of the earlier primordia, is the result of the co-ordinated activation of certain cells and the partial inhibition of division in others; the former become spore producing, the latter are protective.

In ovules in which a single primary hypodermal cell is present the axial row is contiguous to the epidermis, laterally. Where several secondary hypodermal cells produce gynosporocytes some of the latter soon disintegrate, resulting in separation from the remaining cells (Fig. 6). The normal development follows neither of these extremes; several primary hypodermal cells divide periclinally each to form the same number of tiers (Fig. 3); one central group only divides and differentiates to produce the axial row; the other tiers give rise to an adjacent several-layered jacket enclosed by the epidermis. As indicated, the several-layered nucellar wall is partly dermal and chiefly hypodermal in origin.

Normally, the gynosporocyte divides meiotically and produces four gynospores which may be linear in arrangement but more frequently the outer cell, resulting from the heterotypic division, divides transaxially or obliquely. Meanwhile the primary exoparietal cell divides periclinally into two, then three exoparietal cells while the endoparietal cells divide in the transaxial and "ipseaxial" planes, successively (Figs. 7, 8, 9). These divisions are rapid and the resulting columella (basal axis) becomes four to eight cells in length and of four, seldom nine, tiers transversely, that is two, seldom three, cells in each axial plane (Figs. 5 to 8, 10, 12).

At this stage there are variations from ovules with an axial row of four gynospores, two parietals, and four endoparietals (columella) covered by a single-layered epidermis to a much more massive ovule, with 16 gynospores (some deficient), 12 exoparietals, a columella of 90 (±) endoparietals and a several-layered wall of hypodermal origin, covered by the epidermis. Normally, the axial row consists of four gynospores (three deficient and the inner with abundant stored food), two exoparietals, and 24 endoparietals, constituting a columella of four tiers; the hypodermal wall is three cells in thickness near the base and two near the apex; the outer jacket of epidermal cells is single-layered. The primordia of the two integuments form basal rings, adjacent to the columella (Figs. 7, 8).

The Origin and Development of the Specialized Integuments and Funiculus

The Integuments. The two integuments originate as annular ridges or girdles, adjacent to the columella (endoparietal cells) and are almost simultaneous in time of origin (Figs. 6, 7, 8). The most satisfactory basis for designating them is their region of origin and position of attachment, that is as "proximal" (outer) and "distal" (inner). The terms "inner" and "outer" apply to intermediate stages only, while the terms "proximal" and "distal" apply equally at all stages of integumentary development. The distal integument is the product of early periclinal divisions of the dermatogen (outer tunica), followed by rapid anticlinal divisions of the outer layer (Figs. 7 to 10). The resulting extensive two-layered fold (Figs. 10, 12) becomes thickened by periclinal divisions, especially in the region adjacent to the micropyle during the later stages (Figs. 13, 14, 16). The proximal integument is similar except that it has an additional hypodermal (inner tunica) component (Figs. 8, 9) which is most marked at the early stages of growth and which continues until maturity in the adfunicular portion of the flexed ovule to form a massive intrusion resulting from divisions in three planes. The outer protruding lip of the proximal integument originates by anticlinal divisions of the dermatogen and at later stages becomes thickened by periclinal divisions (Figs. 12, 13, 14, 16).

At the archesporial stage the ovule is erect (orthotropous) (Figs. 4 to 7), during meiosis it becomes pendent upon an elongated, terminally flexed funiculus (anatropous) (Figs. 8 to 10), and during the development of the

female gametophyte the ovule itself becomes flexed or geniculate (campylotropous) (Figs. 13, 14). At first the curvature is due to inequalities in the rates of division in different regions of the funiculus (Figs. 9, 10) and later is a result of marked variations in growth rate within the nucellus and the integuments (Figs. 13, 14). For facility of reference the *portion* of the flexed ovule between the micropyle and the funiculus may be designated *endoflexial*, the outer or peripheral portion as *exoflexial* and the wings as *latiflexial* (Figs. 10 to 14, 20 to 22).

The distal integument grows more rapidly in the endoflexial region to form an endoflexial lip, and the proximal integument forms an exoflexial lip in a similar manner, that is, the base of the nucellus becomes enclosed by two integumentary sheathes while the apical portion is protected by a pair of lips, different in origin and development; the distal integument is in contact with the exoflexial region of the nucellar apex, the proximal with the endoflexial region (Figs. 10 to 14, 20 to 22). The micropyle, consequently, is not a cylindrical tube surrounded by the inner integument; it is a doubly crescentic and marginally lobed passage between the exoflexial lobe of the proximal integument and the endoflexial lobe of the distal integument. Median sections at right angles to the plane of flexure show similar latiflexial portions of the distal integument enclosed terminally by the exoflexial lip of the proximal integument (Fig. 12). In this region a section of the micropyle is "I"-shaped. Meanwhile, the endoflexial portion of the proximal integument becomes very much thickened subdermally, and the resulting intrusion is chiefly responsible for the endoflexial curvature of the nucellus and of the embryo sac (Figs. 13, 14).

After the early histogenesis of the integumentary girdles there is a very minor subdermal meristematic activity, except in the endoflexial region of the proximal integument; in this region a mass of cells 20 or more units in thickness is developed by frequent divisions in both periclinal and anticlinal planes, giving rise to a knob-like intrusion into the central region of the flexed ovule. Meanwhile rapid anticlinal divisions of the exoflexial portion of this proximal integument increases the outer surface. The campylotropous ovule is an expression of these modifications of integumentary development (Figs. 13, 14). The great proportion of the integumentary sheathes and terminal lobes originates from the dermatogen. The cells first divide anticlinally to form folds two cells in thickness throughout (Fig. 10); later, secondary divisions, some periclinal, result in more massive portions, especially in the regions of the terminal lips (Figs. 13, 14, 20 to 22).

The notable curvatures of the embryo sac, of the funiculus, of the micropyle, and of other parts of the ovule, about a central massive, endoflexial portion of the distal integument are significant features of development in the campylotropous ovule.

The Funiculus. The nucellus, the integuments, and the axial row, including the sporogenous group and the columella, are entirely tunicate (epidermal and

hypodermal) in origin; in contrast, the *funiculus contains a central region that originates from the primary corpus and develops vascular tissue.*

The archesporial cells and surrounding tissue are embedded (Figs. 1, 2), and the inner cells of hypodermal origin remain below the level of the general surface during the early development of the ovular papillae (Figs. 2, 3, 17), that is, until meiosis begins in the gynosporocyte. During spore production and the development of the functional gynospore, a rapid elongation of the funicular stalk occurs; at *first* the growth is *axially symmetrical*, giving the ovule an erect orthotropous aspect; *later* unilaterally rapid elongation results in a *flexure of the funiculus* and the ovule assumes an anatropous position (Figs. 1, 8, 9, 10, 12, 20, 21). During the development of the female gametophyte there is little or no extension of the funiculus; meanwhile the axial elongation of the nucellus and the development of the integuments, especially the endoflexial portion of the proximal integument, results in the *flexure of the nucellus* and of the embryo sac. The ovule finally takes the infolded or campylotropous posture (Figs. 14, 21, 22).

The histogenesis of the funiculus is distinct from that of the ovule proper (gynosporangium); the flexures and the fusion of parts, however, result in a structural unit that is ordinarily known as the ovule (Figs. 1, 10, 13, 14, 20 to 22). The preovular margins of the infolded carpel are four cells in thickness; periclinal divisions of the hypodermal layer on the abovular side give *five layers* of which *one is central* and may be regarded as *representing the corpus;* on the ovular side, periclinal divisions of the hypodermal layer are suppressed except at the insular regions where repeated periclinal divisions feature development of the gynosporangium. Following the production of an axial row of gynospores and of the exoparietal and endoparietal cells, divisions proceed rapidly, affecting the cells of the corpus adjacent to the basal endoparietals, and thereby the funicular axis is produced. Mitoses are both periclinal and anticlinal; the predominance of the latter results in the axial elongation of the funiculus. Periclinal divisions of the epidermis and hypodermis keep pace and complement the axial development. Ordinarily a single row of spiral vessels is differentiated at the proembryo stage. It is notable that the endoparietal cells of hypodermal origin, which are continuous with the central cells of the funiculus, remain parenchymous, and those adjacent to the embryo sac finally develop gland-like extensions into the antipodal region of the sac (Figs. 13, 14).

Development of the Female Gametophyte

Several investigators, i.e., Martin (16), Reeves (18), and Cooper (7), have described the development of the female gametophyte in *Medicago sativa* L. The development of the British Columbia hybrids follows the same general course, with a few modifications. The gametophyte is *monosporic* (15), originating from the proximal of the four gynospores; the other three disintegrate soon after being formed (Fig. 9). In abnormal ovules more than

one gynosporocyte may produce an additional spore or spores from which temporary gametophytes are initiated. When this occurs it is very evanescent.

The normal female gametophyte finally consists of *eight units;* the egg and two synergids, three antipodals, and two polar nuclei. In the gynospore, three successive free nuclear divisions occur; after the first, the common mass of cytoplasm is dense, spheroid, approximately 40μ in diameter, with abundant starch, with a large central vacuole, and with a somewhat lenticular nucleus at each pole (Fig. 10). After the third nuclear division the central vacuole is still definite in outline but the cytoplasmic mass has increased in volume, its length now being $\pm 70\mu$. Four nuclei are situated at each pole; two nuclei move and approximate each other, lateral to the vacuole; membranes delimit cytoplasmic units around each of the other nuclei while the two median nuclei and the vacuole occupy the residual cytoplasm (Fig. 12). The very rapid elongation of the nucellus and integuments and the enlargement of the *gametophytic chamber* (embryo sac) to a length of $\pm 500\mu$ follows directly. Meanwhile the original vacuole becomes lobed and ultimately forms a network of branched channels; the denser portion of the cytoplasm now occupies the axial region (Figs. 13, 14).

The egg which has finely vacuolate cytoplasm moves to a position proximal to the synergids. The combined synergids form a pear-shaped mass with a median fissure and soon their cytoplasm begins to produce irregular masses of disintegration products; the nuclei are finely granular and inconspicuous and the nucleoli disappear. The polar nuclei fuse near the distal trisect region of the gametophytic chamber (Fig. 14); their apparent movement is explained by the elongation of the sac and the polar interposition of vacuoles; the evidence indicates that the proximal portion of the ovule elongates more rapidly than the distal region and it is suggested that the autokinetic movement of the polar nuclei does not traverse a space much greater than the 20μ that formerly separated the two (cf. Figs. 12, 13). The antipodal cells elongate in accompaniment with the actively elongating sac and appear to invade the proximal third of the gametophytic chamber; they become highly vacuolate and finally disintegrate (Figs. 13, 14). The female gametophyte is now mature.

At the final stage described above one or more male gametophytes invade the gametophytic chamber, the advancing pollen tubes being simple at first, and later becoming branched. The two sperms are elongated; each has spirally-coiled, collateral bands, one of cytoplasm containing refractive globules and the other of densely staining nucleoplasm (Figs. 14, 15). The tube nucleus is very active and approaches the egg. The tube nucleus resembles the nucleus of the egg very closely, and the former may be definitely identified only after repeated observations of the various stages. Its final position in a single pollen tube in the presence of two sperms gives the most direct evidence of its identity (Fig. 15). At this stage the tube nucleus is adjacent to the egg, the zygotic sperm follows, and the other sperm advances

toward the fusion nucleus. The development of the male gametophyte and the process of fertilization are to be described in another paper.

The British Columbia hybrid plants show many deviations from the normal progress of the female gametophyte. The occurrence of two successive free nuclear divisions at the distal pole may result in a four-celled gametophyte, which includes an egg, two synergids, and a remaining cell containing a single polar nucleus which may persist or may disintegrate. Or the normal first mitosis and separation of nuclei may be followed by two mitoses at the distal pole forming the four nuclei, and by one mitosis at the proximal pole forming two nuclei only. In the latter, the development appears normal, except for the limitation of the usual number of antipodals to one. Further description of these phases of arrested development is planned in an account of abnormalities contributing to low seed production.

Development of Nutritive Tissues and Food Storage Tissue

The importance of a balanced food supply and of food utilization by the embryo, the endosperm, and the integuments has been emphasized by Brink and Cooper (2, 3). The sporogenous and gametogenous stages are equally significant, and probably no stage equals the female gametophyte in rate of enlargement and consequent requirement of food material. Food is most readily available in true or in colloidal solution; the most active stages are characterized by vacuolate or finely granular cytoplasm, the interpauses, by stored food products. Alternate stages of food storage and utilization are recognized as featuring progressive periods of growth.

During sporogenesis the columella, composed of endoparietal cells, is the chief medium of communication between the ovule and the carpel wall. The cells of the columella are parenchymous and contain a dense cytoplasm; their development precedes the early gametophyte which partly uses their food store. Later the columella becomes conductive and its glandular basal cells protrude into the gametophytic sac (Figs. 10, 12, 13, 14).

At the time of the first division of the functional gynospore this cell contains an abundant food supply as evinced by the numerous marginal starch grains and granules surrounding the large, central vacuole. The nearly complete reabsorption of the three sister gynospores indicates the source of a considerable proportion of this supply (cf. Figs. 9, 10). Most of the food is utilized during the mitotic division and wall-forming stages of gametophyte development. A very small amount of starch remains in the cytoplasm containing the free nuclei when the six polar cells are delimited. The new cells have a dense, colloidal food-containing cytoplasm and marked nucleoli (Fig. 12).

The rapid enlargement in the gametophytic chamber synchronizes with the partial digestion of the three- to four-layered wall of the nucellus, with the elongation of the columella cells and the proliferation of the gland-like cells into the embryo sac, with the differentiation of conductive tissue in the funiculus, and last, with the disintegration and digestion of the antipodal cells and synergids. The resulting gametophytic chamber is filled with

vacuoles containing soluble food and masses of starch grains, especially in the vicinity of the egg and fusion nucleus (Figs. 13 to 15). The cytoplasm of the egg and of the pollen tube are locally vacuolate or finely granular.

At the time of fertilization the nucellus is entirely disintegrated at the micropylar region whereas one or two cell layers of the antipodal portion usually remain intact. The marked accumulation of stored food within the chamber at the final stage of gametophytic development would appear to originate chiefly from the tapetal-like nucellar jacket and this residual store provides at least partly for the proembryo and early endosperm. The water supply used by the ovule is, probably, derived directly through the columella and the vascular tissue of the funiculus. In view of later developments and competition for the food supply, as emphasized by Brink and Cooper (3), the degree of development of the various parts and their proportionate food supply, at this late gametophytic stage, are significant features in the normal production of seeds. In the British Columbia hybrids the proliferation of the endoflexial portion of the integument normally occurs before the origin of endosperm, and thereby the competition that otherwise would result in "somatoplastic sterility" is reduced or obviated.

Fertilization initiates a series of changes not only in the meristematic development of the embryo and of endosperm cells, but also in the specialization of protective and local storage cells of the integuments (3, 8, and Fig. 16). Normal development of the seed is dependent upon balanced development in these preliminary stages and it is during this critical period that certain of the ovules degenerate most markedly, a significant proportion ceasing development at one of the proembryo stages.

The aim of this account has been to trace the normal development and to designate a standard that may be used in judging the successes and the failures of ovule development in *Medicago*. There are planned further papers that demonstrate the importance of timing in ovule and pollen tube growth, and of the synchronous initiation of zygote and of primary endosperm as factors in the harmonious balance of processes essential for seed production.

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The manuscript has been read with particular reference to their special fields of interest by H. L. Westover, United States Division of Forage Crops and Diseases, R. B. Thomson, University of Toronto, W. P. Thompson, University of Saskatchewan, G. G. Moe, J. Davidson, and V. C. Brink of the University of British Columbia. The writers wish to express sincere appreciation of these favours.

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EXPLANATION OF FIGURES

Figs. 2 to 21 were drawn with the aid of a camera lucida. Figs. 17 to 22 are reconstructions from the analyses of microscopic sections. Figs. 1 to 16 reduced two-thirds in reproduction; Figs. 17 to 22, ca. two-fifths.

LEGEND

(a) Cells of the embryo sac and their precursors, i.e., archesporial cells, gynospores, cells of the female gametophyte, the disintegrating cells of the inner nucellus, cells of the embryo and of the endosperm, all are completely stippled.

(b) Exoparietal and endoparietal (columella) cells of the axial row have nuclei completely stippled and cytoplasm shown by scattered dots.

(c) Cells of the funicular axis (preconductive) have nuclei completely stippled.

(d) Differentiated conductive cells have spiral bands.

(e) Cells of the nucellus have outlines of the nuclei and nucleoli only.

(f) Cells of the distal integument have nuclear outlines only.

(g) Cells of the proximal integument have cytoplasmic membranes stippled.

(h) Cells of the differentiating epidermis, after fertilization, have granules at the inner and outer margins.

(i) All other cells, including peripheral layers of the funiculus, have outlines of walls only.

(j) In Fig. 1 the epiphore (calyx tube or receptacle cup) is shown in outline; the petals have additional marginal stippling; stamens contain pollen grains; the carpel is dotted and the embryo sac is fully stippled; all vascular bundles are enclosed and larger bundles are half-hatched.

DESCRIPTION OF SPECIAL FEATURES OF FIGURES

FIG. 1. *Transverse section of the flower at the time of the two-celled gametophyte, at the anther level of the longer stamens; the folded carpel, with separate funiculus and nucellus of adjacent ovules; five stamens; five filaments (stippled lines) representing the position of the shorter stamens; five separate petals and the epiphore (calyx tube or receptacle cup). 40×.*

FIGS. 2, 3. *Sections of carpel wall at the marginal fold, showing primordia of the ovules at two stages.*

FIG. 2. *General periclinal divisions of the hypodermal layer on contraovular side; localized periclinal divisions giving rise to the secondary gynosporangial (archesporial) cell and the endoparietal initial. 360×.*

FIG. 3. *Several potential gynosporangial tiers, one tier forming the axial row consisting of a tertiary gynosporangial cell (gynosporocyte), the dividing endoparietal cell, and an exoparietal initial. 360×.*

FIGS. 4 to 10. *Sections of the ovular primordium and later the developing ovule, with the funiculus, in the plane of the carpel's longitudinal axis. This plane also shows the flexure of the funiculus and of the ovule and the asymmetrical growth of the integuments.*

FIG. 4. *A single axial row, a gynosporocyte, a single exoparietal, and two endoparietal cells (columella). 360×.*

FIG. 5. *Similar, with three endoparietals. Lateral tiers of potential archesporial cells are forming the inner nucellus. 360×.*

FIG. 6. *Three tiers of active archesporial cells each with a sporocyte, an exoparietal, and three or four endoparietal cells. 200×.*

FIG. 7. *Similar, the secondary sporocyte stage; axial row somewhat asymmetrically situated; primordia of the distal integument, derived chiefly from dermatogen (outer tunica). 360×.*

FIG. 8. *Primordia of proximal integument, partly hypodermal in development; second meiotic division; axial and transverse divisions of the endoparietal cells produce a sectionally double-tiered columella. A 90-degree funicular flexure results in a pendent ovule. 360×.*

FIG. 9. *Similar, and somewhat more advanced; the two proximal gynospores, with dense protoplasm, and axially situated; the two distal gynospores (one shown), disintegrating and obliquely placed. 360×.*

FIG. 10. *A funicular flexure through 180 degrees results in an anatropous ovule; the exoflexial extension of the distal integument is marked; a binucleate female gametophyte, with large central vacuole. 360×.*

FIG. 11. *A tetranucleate female gametophyte. 430×.*

FIG. 12. *A section of the nucellar portion of an ovule (funiculus omitted) in a plane transverse to the flexed axis of the carpel (cf. Fig. 1). The compacted, seven-celled stage of the female gametophyte: egg, two synergids, three antipodals, central cell containing two polar nuclei, the vacuole extending and branching. The exoflexial fold, only, of the distal integument appears. 360×.*

FIGS. 13, 14. *Sections of the ovule in the plane of the longitudinal axis of the carpel showing the flexures of the campylotropous ovule.*

FIG. 13. *A greatly elongated gametophytic sac occasioned by the anticlinal divisions of the wall cells, especially the exoflexial. Micropyle surrounded by the exoflexial lobe of the proximal integument and the endoflexial lobe of the distal integument; the distal integument enclosing the basal portion of the nucellus only, on the exoflexial side; the proximal integument becomes massive at the centroflexial region. Funiculus with vascular strand; glandular, hair-like endoparietals; exoparietals lenticular; disintegrating nucellar cells; antipodals suspended in the sac; synergids disintegrating; polar nuclei approximated; tube nucleus in contact with the egg. 165×.*

FIG. 14. *Similar and further developed, immediately preceding fertilization. Distal portion of nucellus completely disintegrated; fusion nucleus; rich food store and extreme vacuolation within gametophytic sac. Spiral sperm lateral to synergids. 165×.*

FIG. 15. *The pollen tube, containing the tube nucleus and two spiral sperms, advancing by the side of the egg, with lobes approaching the fusion nucleus (latter not shown); synergids disintegrating; cytoplasm of egg and pollen tube vacuolate and surrounded by abundant starch. Nuclei of egg and tube containing densely staining nucleoli. 930×.*

FIG. 16. *Section of micropylar portion of the ovule, transverse to the longitudinal axis of the carpel; the filamentous proembryo and free nuclear endosperm. Epidermis of the proximal integument differentiated and containing abundant globules, the distal integument and nucellus discontinuous, distally. 165×.*

FIGS. 17 to 22. *Diagrams to illustrate the development of the campylotropous ovule of Medicago, and particularly the relationships of the funiculus, the integuments, the nucellus, and the gametophytic sac. Figures on the left are "translux" diagrams, on the right are surface diagrams, except that the positions of the nucellus, the gametophytic sac, and the vascular bundle are indicated by series of dots. ca. 90×.*

FIG. 17. *The ovular mound or primordium.*

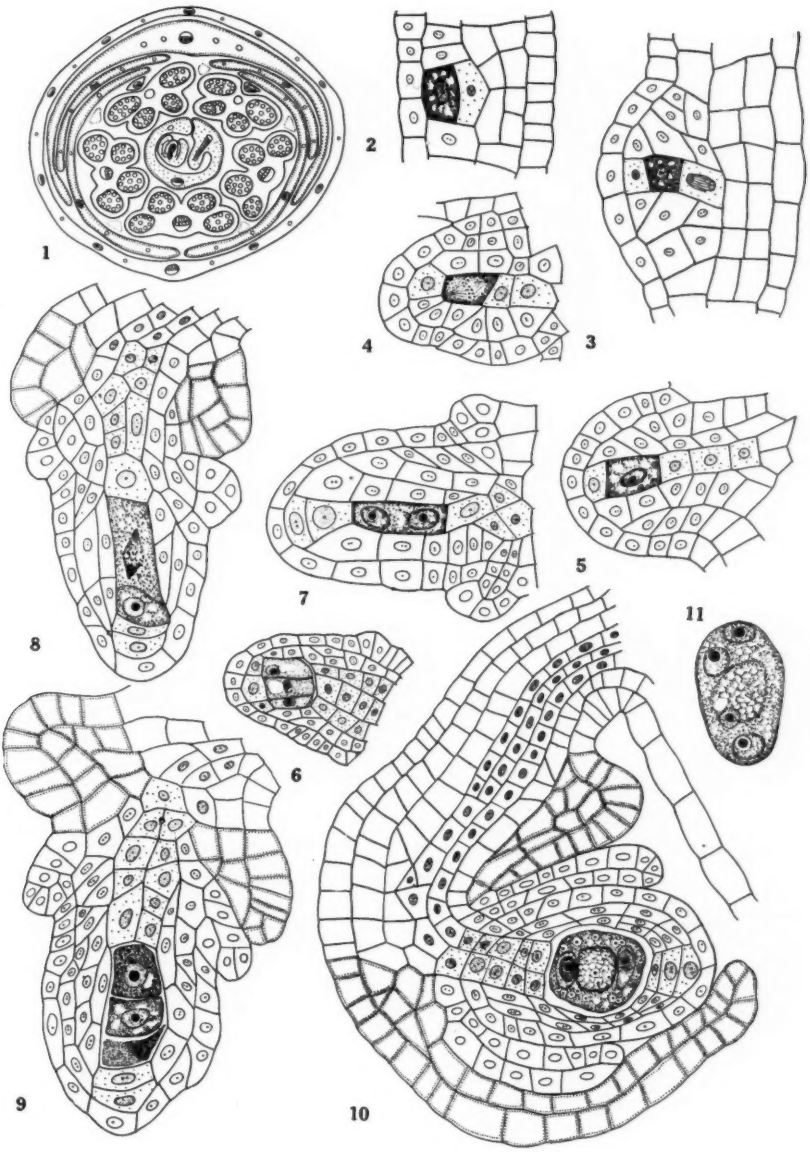
FIG. 18. *Nucellus exposed; funiculus elongating; distal integument initiated; axial row, including gynospores, developing; columella complete.*

FIG. 19. *Flexure of funiculus begins, proximal integument forms an exoflexial lip, proximal gynospore differentiates.*

FIG. 20. *Funicular flexure, 135 degrees, exoflexial lip of the proximal integument and endoflexial lip of the distal integument nearly enclose the nucellus; seven-celled, female gametophyte retains the central vacuole and ovoid form.*

FIG. 21. *The arc flexure of the funiculus complete; the vascular strand differentiating; the integumentary lips enclose the nucellus except for a sectionally doubly-crescentic micropyle; the exoflexial lip of the proximal integument nearly encloses the lip of the distal integument; the endoflexial portion of the proximal integument becomes massive and occasions the flexure of the elongating gametophytic sac; female gametophyte mature; glandular development of distal cells of columella.*

FIG. 22. *The sigmoid flexure of the funiculus accompanies a marked proliferation of the proximal integument in the centroflexial region; the filamentous proembryo and the free nuclear endosperm invade the crescentic embryo sac of the campylotropous ovule.*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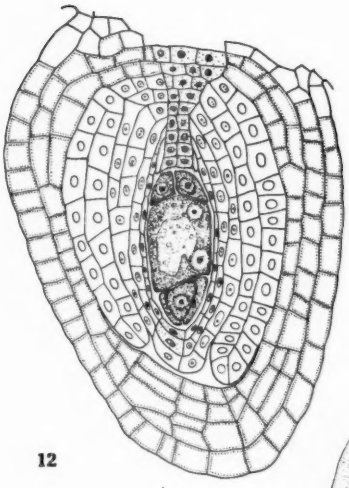

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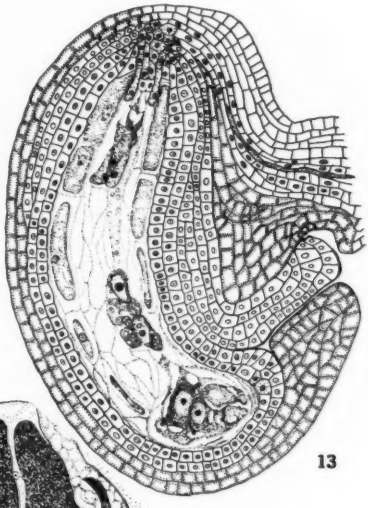

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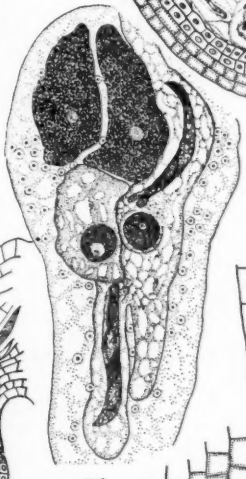

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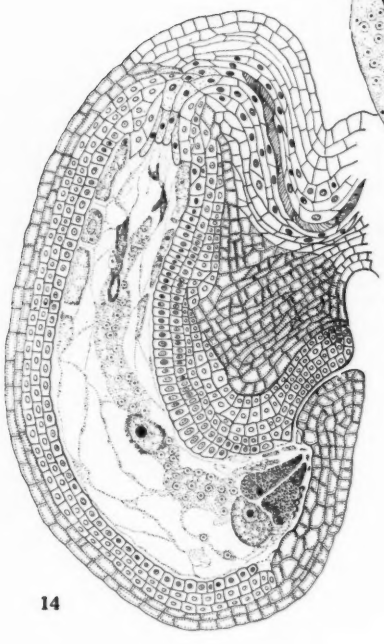

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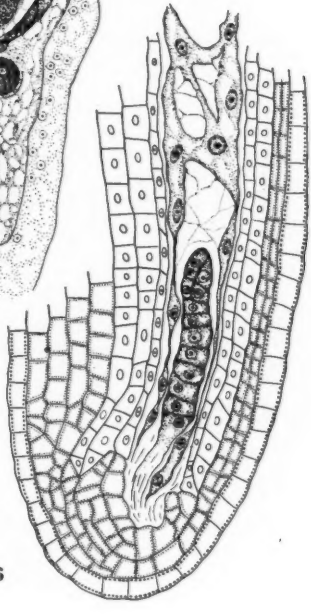

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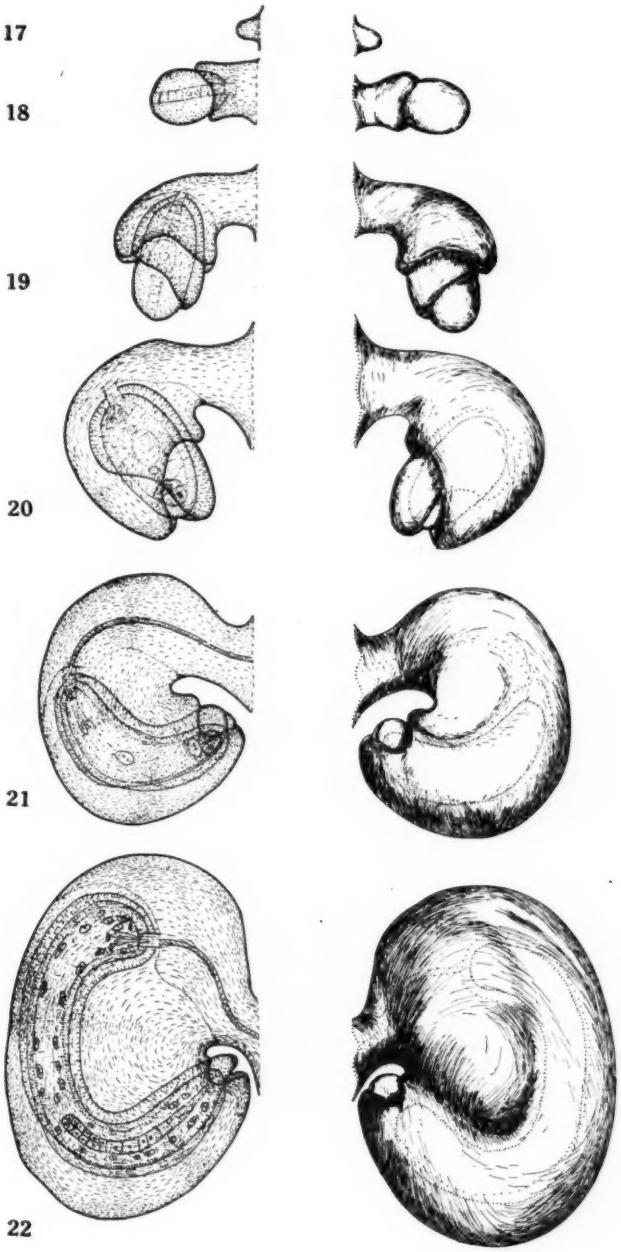
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THE EFFECT OF HIGH TEMPERATURE ON THE STEM RUST RESISTANCE OF WHEAT VARIETIES[1]

By T. Johnson and Margaret Newton[2]

Abstract

Eighteen stem rust resistant wheat varieties were tested, in the greenhouse, for their reaction to three physiologic races of *Puccinia graminis Tritici* Erikss. and Henn. at three different temperatures: a constant low temperature of about 60° F., a constant high temperature of about 80° F., and an intermediate temperature which fluctuated daily from 50° to 55° F. at night to 70° to 85° F. at midday.

At the low and at the intermediate temperature some of the varieties proved immune while others proved highly or moderately resistant. At the high temperature five varieties (Bokveld, Iumillo, Gaza, Red Egyptian, and N.A. 95 Egypt) were immune or highly resistant; six varieties (Marquillo × Waratah, Hope, Hochzucht, Minor, Bobin Gaza Bobin, and Federation × Acme) were moderately resistant; and seven varieties (Kenya, Syria, McMurachy, Sweden, Rhodesian, Talberg, and Eureka) were moderately or completely susceptible.

Introduction

It is well known that temperature influences the stem rust reaction of the wheat plant. This fact has been clearly demonstrated by several investigators in studies on the rust reaction of wheat seedlings. Waterhouse (11) and Johnson (5) showed that the indeterminate (x) reaction which certain varieties display at moderate temperatures is replaced by resistance at low and susceptibility at high temperatures. Melander (8) has shown that temperatures slightly above freezing largely suppress stem rust development and Johnson and Newton (6) have shown that excessively high temperatures induce resistance, at least to some physiologic races.

Less attention has been given to the influence of temperature on the stem rust reaction of the wheat plant in more advanced stages of growth. It has, however, been shown recently (9, 10) that certain strains of *vulgare* wheat from Kenya, East Africa, and the Canadian variety McMurachy, which are immune or nearly so at low and moderate temperatures, become partially or completely susceptible at high temperatures in both the seedling and the adult stage. Somewhat similar results had previously been reported for the variety Hope (7) which proved less resistant at 75° to 80° F. than at lower temperatures. The studies on the influence of temperature on reaction to stem rust were extended to include a group of wheats from a large collection made by Mr. S. L. Macindoe of Sydney, Australia. The varieties tested were selected from a larger number grown in 1940 on the plots of the Dominion Rust Research Laboratory where they were exposed to natural infection

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plus an artificially produced stem rust epidemic. In addition to the varieties selected from Mr. Macindoe's collection, there were also included in these tests the resistant varieties McMurachy, Kenya R.L. 1373, Hope, and Iumillo, and, for purposes of comparison, the susceptible variety Marquis. With the exception of Marquis and Eureka, all the varieties selected for the temperature studies had shown immunity or near-immunity in the field tests.

The Kenya strains and McMurachy have, since they were first grown at Winnipeg in 1934 and 1935, respectively, proved immune to stem rust or nearly so. In 1938, it was brought to the attention of the plant breeders at Winnipeg that these varieties were not immune and sometimes not even highly resistant when grown at St. Paul, Minn., Manhattan, Kansas, and some other points in the United States. It seemed probable therefore that their rust reaction was being influenced by the environment in which they were grown. The first proof that their resistance disappeared at high temperatures came as a result of experiments conducted by the writers in an attempt to explain certain unexpected infection results obtained in progeny tests carried out by Dr. R. F. Peterson in the winter 1939–1940. He found that seedlings of Kenya, McMurachy, and some of their progeny, known to be immune in the field, proved at times to be only moderately resistant or even susceptible when tested in the greenhouse. At his request an investigation was undertaken. Experiments in which light and temperature were kept under control soon proved that high temperature was the chief factor responsible for the breakdown of seedling resistance. Subsequent experiments, as already mentioned, have shown that the resistance of adult plants may also be broken down at high temperatures.

Methods

Three physiologic races with distinctly different infection characteristics were selected for the tests, namely, races 56, 29, and 15. Race 56 is, at present, the predominant physiologic race of wheat stem rust in North America. It attacks many *vulgare* wheats severely, but is not highly pathogenic on most *durum* and emmer wheats. Race 29 attacks many *vulgare* wheats heavily, *durum* wheats moderately, but emmer wheats lightly. Race 15 attacks all three groups of wheat rather severely.

The test plants were grown to the heading stage in 6-in. flower pots—four plants to each pot. At heading or immediately after, the inoculum was applied to leaves, stems, and peduncles with the fingers, after which the plants were kept for 24 hr. in damp chambers consisting of a framework of wooden laths covered with heavy cardboard which, when soaked with water, maintained a satisfactory film of moisture on the plants. Infection took place at a moderate temperature, but when the plants were removed from the inoculation chamber four plants inoculated by each physiologic race were placed in each of three greenhouses maintained at different temperatures. Two of these greenhouses were thermostatically controlled. These were kept respectively at around 60° and 80° F. In the third greenhouse the temperatures fluctuated daily from 50° to 55° F. at night to 70° to 85° F. at

TABLE I

REACTION IN GREENHOUSE TESTS, OF 19 WHEAT VARIETIES IN THE HEADING STAGE TO THREE PHYSIOLOGIC RACES OF WHEAT STEM RUST AT LOW, MEDIUM, AND HIGH TEMPERATURES (RUST PERCENTAGES IN PARENTHESES)

Variety	1st Test—December to March							
	Date inoc.	Temp., °F.	Race 15		Race 29		Race 56	
			Leaves	Sheaths	Leaves	Sheaths	Leaves	Sheaths
Iumillo R.L. 7*	Jan. 27	60.3 63.5 81.5	I – –	I – –	I – I	I – R	I I I	I I I
Gaza W.L.W.–S. 755**	Dec. 2	61.3 63.1 81.0	I – I	I – I	I – I	I – I	I I I	I I I
Bokveld C. 12080–S. 656								
Red Egyptian C. 12081–S. 979	Jan. 17	61.2 60.9 82.6	I – R	I – R	I – R	I – R	I I R	I – R
N.A. 95 Egypt C. 12095–S. 674	Jan. 9	60.1 61.0 83.2	– – R	I – R	I – R	– – I	I I R	I – R
Marquillo × Waratah S. 906	Dec. 6	61.7 63.7 82.3	I – I	I – –	I – I	I – I	I I I	I I I
Hope R.L. 209	Feb. 12	60.5 64.2 80.6	MR – MR	MR – MR	R – MR	MR – MR	R I R	I I I
Hochzucht C. 10847–S. 765	Jan. 22	60.2 72.8 80.7	R – R	R – R	R – I	R – I	I I MR	R I MR
Minor C. 9142–S. 931	Feb. 13	60.8 63.9 80.4	R – R	R – R	R – MR	MR – MR	– R MR	– R R
Bobin Gaza Bobin S. 203								
Federation × Acme S. 630	Feb. 4	62.4 62.4 80.2	R – R	R – R	R – R	R – R	R R R	R R R
Syria C. 10349–S. 1027	Feb. 18	60.6 64.9 80.7	I – MS	I – MS	I – MS	I – S	I I MS	I I S
McMurachy R.L. 1313	Jan. 17	61.2 63.1 82.9	I – –	I – –	I – S	R – S	I I MS	I – S
Kenya R.L. 1373	Feb. 3	59.5 64.7 81.0	I – MS	I – S	I – MS	I – MS	I I MR	I I MS
Sweden C. 12266–S. 1038	Jan. 8	60.3 60.7 83.0	I – MS	I – MS	– – S	– – MS	I I S	I R MS
Rhodesian TRS No. 8–S. 971	Jan. 23	60.1 72.7 80.7	I – S	I – S	I – S	I – S	I I S	I I S
Talberg C. 12261–S. 1056	Feb. 7	60.0 63.5 80.0	I – S	I – S	I – S	I – S	I I S	I I S
Eureka 2 C. 12890–S. 723								
Marquis R.L. 572	Jan. 29	61.0 63.4 82.1	MS – S	S – S	S – S	S – S	MS S S	S S S

NOTE. I = *immune*; R = *resistant*; MR = *moderately resistant*; MS = *moderately susceptible*; S = *susceptible*; tr = *trace*.

* *Dominion Rust Research Laboratory accession number.*

** *Number under which the variety was received from Mr. S. L. Macindoe.*

TABLE I—*Concluded*

REACTION IN GREENHOUSE TESTS, OF 19 WHEAT VARIETIES IN THE HEADING STAGE TO THREE PHYSIOLOGIC RACES OF WHEAT STEM RUST AT LOW, MEDIUM, AND HIGH TEMPERATURES (RUST PERCENTAGES IN PARENTHESES)—*Concluded*

Variety	2nd Test—March to May							
	Date inoc.	Temp., °F.	Race 15		Race 29		Race 56	
			Leaves	Sheaths	Leaves	Sheaths	Leaves	Sheaths
Iumillo R.L. 7*	April 25	63.4 69.2 78.4	I I I	I I I	I I I	I I I	I I I	I I I
Gaza W.L.W.–S. 755**	Mar. 18	61.5 66.7 81.3	I I I	I I I	I I I	I I I	I I I	I I I
Bokveld C. 12080–S. 656	May 1	68.3 69.9 77.2	I I I	I I I	I I I	I I I	I I I	I I I
Red Egyptian· C. 12081–S. 979	April 24	65.5 68.9 78.4	I I R(tr)	I I R(10)	I I R(tr)	I I R(tr)	I I R(tr)	I I I
N.A. 95 Egypt C. 12095–S. 674	Mar. 27	63.5 66.6 80.2	R(tr) R(tr) R(tr)	I R(tr) R(tr)	R(tr) R(tr) R(tr)	R(tr) R(tr) R(tr)	R(tr) R(tr) R(tr)	R(tr) R(tr) R(tr)
Marquillo × Waratah S. 906	Mar. 24	64.2 64.5 83.6	R(tr) I MR(tr)	MR(tr) I MR(tr)	I I I	I I I	R(tr) I MR(3)	R(tr) R(tr) MR(1)
Hope R.L. 209	April 28	62.6 67.9 78.8	MR(tr) R(tr) MR(tr)	MR(20) MR(15) MR(15)	MR(tr) R(tr) I	MR(5) MR(10) MR(15)	I I I	I R(tr) I
Hochzucht C. 10847–S.765	April 15	64.7 68.2 78.9	MR(30) R(20) MR(10)	MS(50) MR(40) MR(25)	R(5) R(tr) R(tr)	R(5) I I	R(1) R(5) R(5)	MR(5) MR(25) MR(5)
Minor C. 9142–S. 931	May 1	68.3 69.9 77.2	MR(25) R(25) R(20)	MR(30) R(35) MR(20)	MR(15) R(15) MR(15)	MR(40) MR(30) MR(35)	MR(20) R(25) MR(35)	MR(30) R(40) MR(45)
Bobin Gaza Bobin S. 203	Mar. 27	63.3 66.7 83.4	MR(20) MR(25) MR(15)	MR(25) MS(50) MR(45)	MR(20) MR(20) MS(25)	MR(35) MS(45) MS(50)	R(15) MR(25) MR(20)	R(25) MR(50) MR(40)
Federation × Acme S. 630	April 22	64.6 68.3 80.8	R(5) MR(5) MR(5)	R(25) MR(20) MS(20)	R(tr) MR(5) MR(2)	R(10) MR(25) MS(15)	I R(10) MR(1)	R(tr) MR(20) MR(10)
Syria C. 10349–S. 1027	May 2	69.1 71.5 76.3	I I MR(5)	R(5) R(tr) MR(45)	I I R(tr)	R(2) R(tr) MS(25)	I I I	R(tr) R(tr) MR(10)
McMurachy· R.L. 1313	April 29	65.8 69.3 80.3	R(tr) R(2) MS(5)	R(tr) MR(20) MS(10)	R(tr) I S(5)	MR(tr) R(2) S(10	R(tr) R(2) MS(3)	R(tr) MR(15) MS(2)
Kenya R.L. 1373	April 28	62.6 67.9 78.8	I R(2) MS(10)	I R(10) MS(40)	I MR(tr) MS(10)	I R(tr) MS(25)	I R(tr) MR(5)	I R(5) MR(15)
Sweden C. 12266–S. 1038	April 21	63.7 67.9 78.6	I MR(10) MS(2)	MR(tr) MR(25) MS(20)	I R(5) S(10)	R(tr) MR(20) S(45)	I R(1) MS(15)	R(tr) MR(15) MS(30)
Rhodesian TRS No. 8–S. 971	April 23	64.3 69.0 79.5	I R(tr) S(2)	I R(tr) S(10)	I I S(2)	I R(tr) S(10)	I R(tr) S(2)	I R(tr) S(5)
Talberg C. 12261–S. 1056	April 29	65.8 69.3 80.3	R(tr) MR(3) S(5)	R(tr) MS(15) S(10)	R(tr) R(tr) S(tr)	MR(5) R(tr) MS(5)	MR(tr) R(tr) S(tr)	MR(tr) MR(5) S(1)
Eureka 2 C. 12890–S.723	Mar. 27	63.3 66.7 84.3	R(2) MR(5) MS(30)	MR(5) MR(25) S(60)	I R(tr) S(40)	R(tr) MR(3) S(65)	I MR(5) MS(30)	R(tr) MR(20) S(80)
Marquis R.L. 572	April 24	65.5 68.9 78.4	S(20) S(25) S(10)	S(50) S(70) S(50)	S(20) S(25) S(25)	S(55) S(50) S(55)	S(25) S(30) S(30)	S(65) S(65) S(60)

NOTE. I = *immune;* R = *resistant;* MR = *moderately resistant;* MS = *moderately susceptible;* S = *susceptible;* tr = *trace.*

* *Dominion Rust Research Laboratory accession number.*

** *Number under which the variety was received from Mr. S. L. Macindoe.*

midday. It should be noted that the mean daily temperatures expressed in Table I were calculated from thermographic records at two-hour intervals.

Two successive tests were carried out on all but three of the 19 varieties studied. The first test, on plants sown September 20, 1940, was made during the winter months of December, January, February, and early March. The second test, on plants sown January 10, 1941, was conducted from the latter part of March until about the middle of May. Towards the end of this period (particularly from May 1 to May 20) the out-of-doors temperature became too high for satisfactory thermostatic control of the low temperature greenhouse. The mean daily temperature of this greenhouse therefore rose considerably towards the end of the second test.

The tests of the adult plants were accompanied by simultaneous infection tests with seedlings. All varieties were also tested at 60° and 80° F. to eight other physiologic races (races 17, 19, 21, 32, 36, 38, 48, and 152) so that some knowledge could be gained of their general rust reaction.

In estimating the degree of resistance of the adult plants personal judgment was eliminated as far as possible by recording the infection types present on each of the four uppermost leaves and sheaths and converting these into numerical values for which averages for leaves and stems could be calculated. The system used was a modification of that originally described by Goulden, Newton, and Brown (4). The infection types 0, 1, 2, 3, and 4 were given values of 1, 5, 10, 15, and 20, respectively, and the types x=, x−, x, x+, and x++ values of 11, 12, 13, 14, and 15. In determining the rust reaction of a variety, numerical values of 0 to 2.5 were regarded as representing immunity, 2.6 to 7.5 resistance, 7.6 to 12.5 moderate resistance, 12.6 to 15.5 moderate susceptibility, and 15.6 and above as susceptibility.

The Effect of Temperature on Stem Rust Reaction

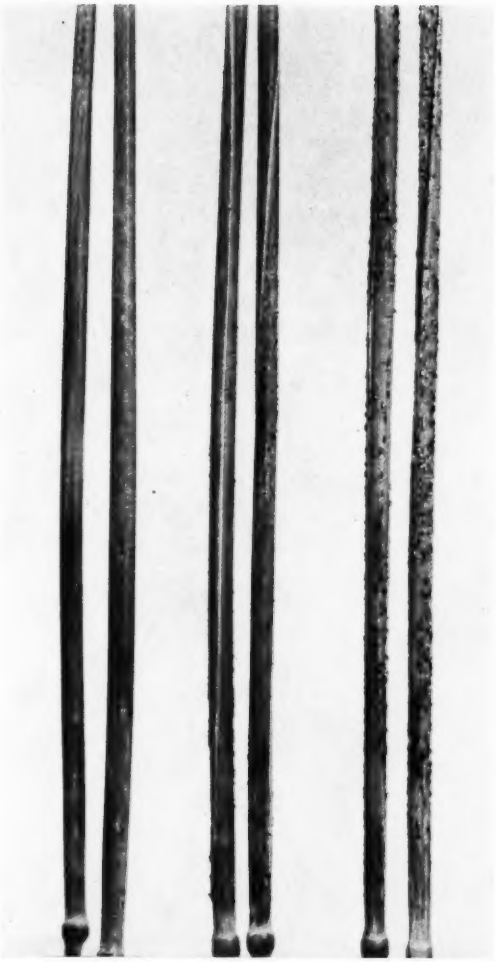
Field Test

As already stated, all the varieties tested in the greenhouse were subjected, in the summer of 1940, to a field test in which they were exposed to natural stem rust infection on which was superimposed an artificially induced epidemic comprising many physiologic races. Of the 19 varieties previously mentioned only two, Marquis and Eureka 2, were severely rusted, four (Gaza, Rhodesian, Kenya, and Iumillo) bore no stem rust infection, while the remaining 13 varieties showed only a trace of rust.

Greenhouse Tests on Adult Plants

The two tests with adult plants recorded in Table I were conducted under approximately the same conditions of temperature. Conditions of light, however, were not similar owing to the fact that the first test was conducted in midwinter, whereas the second was carried out in the spring. It should be

PLATE I



Reaction of the uppermost sheath of Eureka 2 to P. graminis Tritici race 56 at three different temperatures: left, at 63.3° F.; centre, at 66.7° F.; right, at 84.3° F.
At fluctuating temperatures the largest pustules are directly above each node.

noted, too, that the first test was less comprehensive inasmuch as only race 56 was used in the infection studies at moderate (fluctuating) temperature.

Table I gives, for each variety, the mean reaction of leaves and sheaths as determined by infection type and also, for the second test, the amount of rust, expressed in percentages. The percentage figures, as well as the reaction symbols, are indicative of the degree of resistance, but undue emphasis should not be placed on the percentages because of possible variation in the amount of inoculum applied and conditions of temperature and moisture at the time of infection.

On the basis of response to temperature the varieties may be divided into two groups: those whose reaction is little influenced by temperature, including Gaza, Bokveld, Iumillo, Red Egyptian, N.A. 95 Egypt, Marquillo × Waratah, Hope, Hochzucht, Minor, and Bobin Gaza Bobin; and those whose resistance is more or less broken down at high temperatures, including Federation × Acme, Syria, McMurachy, Kenya, Sweden, Rhodesian, Talberg, and Eureka. The last six varieties were the ones most markedly affected by temperature. Marquis, which was included in the tests only for comparison, was equally susceptible at all three temperatures. Within each of these two groups the varieties are arranged in Table I in approximate order of resistance. Plate I shows the reaction of Eureka at three different temperatures.

A few discrepancies between the first and second tests may be noted. Marquillo × Waratah proved immune at all three temperatures in the first test but was in some cases only moderately resistant in the second test. Hochzucht was somewhat less resistant in the second test than in the first, and Federation × Acme was more susceptible at the high temperature in the second test. In most of the varieties, however, agreement between the two tests is reasonably close.

Greenhouse Tests on Seedling Plants

All the varieties except Bokveld were tested in the seedling stage at 60° and 83° F. for their reaction to physiologic races 15, 17, 19, 21, 29, 32, 36, 38, 48, 56, and 152. In general, the seedling response to temperature of each variety resembled that of the adult plants closely. Two varieties, however, proved exceptional, namely, Marquillo × Waratah, whose resistance to races 15 and 56 broke down at the high temperature in the seedling stage but not in the adult stage, and Minor, whose seedlings were somewhat more susceptible than the adult plants at both temperatures. The variety Hope differed from the other varieties tested in that it was susceptible in the seedling stage to several races to which it has mature-plant resistance.

At the low temperature the following varieties proved either immune or highly resistant to the eleven above-mentioned races: Iumillo, Gaza, Bobin Gaza Bobin, Eureka, Red Egyptian, Sweden, Kenya, McMurachy, Federation

× Acme, Rhodesian, Syria, and Talberg. N.A. 95 Egypt proved moderately resistant to all the races, while Minor was moderately resistant to some and highly resistant to others. Hochzucht and Marquillo × Waratah were susceptible to races 36, 38, and 48 but resistant to others. Hope was susceptible to races 15, 17, 21, 29, and 32.

Discussion

It has become clear within the last few years that stem rust resistance of wheat varieties varies considerably according to the locality in which the varieties are grown. Those that are resistant when grown in the Great Plains region of North America are not necessarily so when grown in South America (1) or in Africa (2, 3). Such discrepancies in reaction may be due to the presence of pathogenically different strains of the rust in different regions, or they may result from differences in environmental conditions, or from a combination of both factors.

Data presented in Table I show that varieties that at low or moderate greenhouse temperatures possess about an equal degree of resistance to stem rust may vary greatly in their stem-rust reaction at higher temperatures. Some, like Iumillo, Bokveld, Gaza, and Red Egyptian, maintain much or all of their high resistance at higher temperatures; others, including McMurachy, Kenya R. L. 1373, Talberg, Sweden, Rhodesian, Syria, and Eureka 2, lose most or all of their resistance when the mean temperature approaches 80° F. In the field, the last-mentioned group of varieties could not be expected to display high stem rust resistance in regions where temperatures are excessively high during the period of stem rust infection. McMurachy and the Kenya wheats are, in fact, known to have less stem rust resistance at St. Paul, Minn., and at Manhattan, Kansas, than at Winnipeg. The summer temperatures at these points are definitely higher than at Winnipeg. For the 9-year period 1932–1940 the mean temperature for July was 69.7° F. at Winnipeg, 76.7° F. at St. Paul, and 84.2° F. at Topeka, Kansas (50 miles from Manhattan). The mean temperature at Winnipeg is below that which, according to greenhouse experiments, should induce susceptibility (about 75° F.). At St. Paul the temperature is approximately at the point where some breakdown of resistance might be expected, while at Topeka, Kansas, it is definitely of the order required to bring about susceptibility. The reaction of the Kenya wheats at St. Paul in 1940 may provide a specific instance of the influence of high temperature on rust reaction. According to Watson (12), some wheat varieties including certain of the Kenya wheats were less resistant at that station than usual. In that year, the mean temperature for July was 75.8° F., whereas the mean temperature for the last 15 days of July was about 81° F. It seems probable that the unusually high temperatures that prevailed in the latter part of July influenced the stem rust reaction of the Kenya wheats.

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A *PHYTOPHTHORA* TOMATO DISEASE NEW TO ONTARIO[1]

By Lloyd T. Richardson[2]

Abstract

In recent years a destructive disease has affected tomato plants in certain greenhouses in central Ontario. This disease is characterized by a damping-off of seedlings and a rot of stems, fruits, leaves, and roots. The causal organism has been identified as *Phytophthora parasitica* Dast. The effects of various nutrient media, of temperature, of atmospheric humidity, and of the acidity of the medium on the growth of the fungus in culture have been studied.

An extensive investigation has been made of certain factors affecting the parasitic activity of this soil-borne pathogen. Disease is favoured by high atmospheric temperature and high relative humidity. Disease incidence also varies directly with the moisture content of the soil and is maximum at soil temperatures near 22° C. The ability of the pathogen to establish itself in soils depends upon the type of soil, upon the other micro-organisms present, and upon the substrate available for saprophytic development. The rates of invasion of non-infested soils were determined and found to be retarded by the competitive factor and accelerated by the presence of living roots of tomato seedlings. The activity of the pathogen is suppressed by the addition of soybean residue to infested soil.

The potential host range of this isolant of *P. parasitica* has been partially determined by means of artificial inoculations.

Several methods for the control of the disease were tried, and their value is discussed.

Introduction

Within recent years there has appeared in central Ontario a destructive disease affecting tomato plants in all stages of their development. This disease is not only new to this area, but seems to be distinct from any other reported on this host. Thus far the outbreaks have been strictly localized in distribution and confined almost exclusively to tomato plants grown under glass. The disease was first observed in the Experiment Station greenhouses at Vineland, Ontario, during the summer of 1937. Since then it has appeared in several of the greenhouses in this district and in one outdoor crop. Losses in some instances were very heavy.

In addition to these cases, which were confined to central Ontario, Dr. Fitzpatrick of the Dominion Laboratory of Plant Pathology at Summerland, B.C., reported in 1940 one outbreak of a similar disease in the Okanagan Valley. A comparison of the causal organisms showed the two to be identical.

The fact that new cases are reported each year seems to indicate that the disease is increasing in economic importance. Since this is the case and since there is no information available regarding the disease, it is considered desirable that the results of this investigation should be reported at the present time.

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Contribution from the Department of Botany, University of Toronto. Based on a thesis presented in May, 1941, to the University of Toronto in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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The investigation covered several aspects of the disease. The causal organism was isolated and identified as *Phytophthora parasitica* Dast. The effects of various factors on the growth of the organism in culture were studied. With respect to epidemiology, extensive studies were made of the factors influencing the parasitic activity of the pathogen. Its ability to attack species of plants other than its natural host was also investigated. Finally, possible methods for the control of the disease were tested.

Symptoms of the Disease

The most striking aspect of the disease is its attack on the seedling stage of the host. Seedlings may be killed either before or after they emerge from the soil. The amount of pre-emergence blight can be determined by comparing the percentage germination of seeds planted in non-infested soil of the same type. Postemergence killing of seedlings results in the effect commonly called "damping-off". A seedling may be killed while emerging, before the hypocotyl becomes erect and pulls the cotyledons out of the soil. If it is attacked later, localized killing of the tissues occurs, usually near the soil line, and the plant falls over. Young seedlings collapse so quickly that several in a flat may fall over within an hour, and, under conditions favourable for disease, 50% may succumb in a single day. Seedlings that are growing in a flat of infested soil become infected not only when they are young, but continue to die, though at a decreasing rate, as they become older. When the survivors are transplanted, even into non-infested soil, there is a very high rate of mortality, amounting in some cases to 100%.

In older plants grown indoors infection is largely confined to the stems. The symptoms here might be described variously as a collar rot, stem girdle, or stem canker (Plate I, Figs. 10, 11, and 13). While in many cases the lesion is restricted to a narrow zone that girdles the stem, in other cases it extends along the stem for several inches before the plant succumbs. On staked plants growing in the greenhouse the lesions are invariably located within a foot of the ground, or in other words they are confined to the region where there is opportunity for infested soil to be splashed in watering. The stem appears brown in colour and somewhat constricted over the area covered by the lesion. It also feels spongy due to the destruction of the parenchymatous tissues. If the stem is cut open at this point (Plate I, Fig. 11), only the woody vascular tissues will be found intact.

The variation in the appearance of the disease on the stem may be related to the age of the plant and its rate of growth, and also to environmental conditions. Occasionally the lesions cease to advance before girdling is complete. The plant may then recover, forming cork over the lesion. Young actively growing parts of the stem tend to be more susceptible than older, hardened parts. A pot-bound plant, inoculated by placing a drop of zoospore suspension on each internode and kept in a moist chamber for a few days, will show symptoms of disease only at the uppermost three or four internodes. If soil is piled around the stem of a plant with a basal lesion, the plant may

form adventitious roots above the lesion and survive. On the other hand the lesion may continue up the stem, especially if the tissues are young, and death is merely delayed by the treatment. Under very humid conditions lesions tend to extend further along the stems than they do in a drier atmosphere.

The foliage of the host is affected both directly and indirectly by the disease. In mature plants, usually the first sign of disease that is noticed by the grower is a sudden wilting of the whole plant. This is a secondary effect resulting from the destruction of the stem at a basal lesion. Such wilting does not occur for some time after girdling is complete, so that the foliage of non-staked plants may appear fresh even when the stem can no longer support it. This suggests that the conducting tissue is the last to be destroyed. Wilting of individual leaves occurs also as a result of infection of their petioles. Direct infections are found only on the basal leaves of staked plants, although many natural infections were found on the leaves of non-staked plants growing in infested soil in the field. Such infections kill the tissues, resulting in irregular brownish patches on the leaflets. Leaves, inoculated with drops of zoospore suspension then placed in a moist chamber, show brown lesions within 24 hours. Plate I, Fig. 12, shows leaves three days after such an inoculation. Under these conditions the necrotic areas continue to enlarge until the whole leaf is involved.

Fruits in contact with the soil, or near enough to it to become splashed in watering, develop characteristic lesions which spread until the whole fruit is decayed. The surface of the fruit over the diseased area is smooth and not sunken. The lesion has a dark brown centre surrounded by an advancing zone that has a grayish water-soaked appearance. Both green and ripe fruits may be attacked, and the lesion may extend deeply into the tissues (Plate I, Figs. 14 and 15). Infected fruits placed in a moist chamber become covered within a day or two with a fluffy mass of mycelium bearing sporangia.

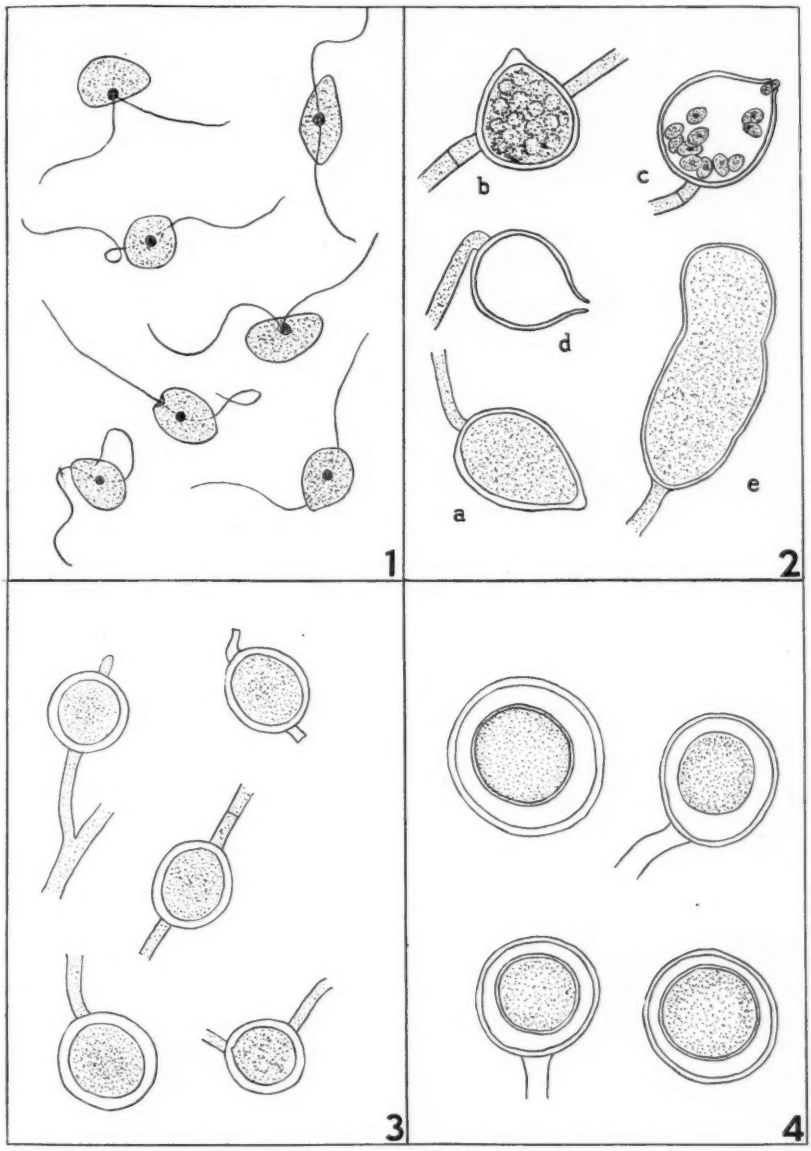
Roots of seedlings grown in infested soil show rotted portions, and mycelium may be observed within the tissues. While it is difficult to demonstrate infection on the roots of mature plants, it is probable that the vigour of all plants growing in infested soil is reduced by such attacks on the root system.

Etiology

The rules of proof of pathogenicity have been applied to the organism causing this disease. It has been found invariably associated with all phases of the disease; it has been repeatedly isolated from diseased tissues and cultured on artificial media; when healthy tissues were inoculated with the fungus from these pure cultures the same symptoms were produced, and the same fungus was recovered on isolation.

A. Description of the Causal Organism

Infected fruits or stems kept in a moist chamber soon develop an abundance of white, cottony mycelium. Abundant aerial mycelium is also produced on artificial media, especially oatmeal agar, on which it becomes matted and



FIGS. 1 TO 4. *Camera lucida drawings of various organs produced by P. parasitica in culture on oatmeal agar.*

FIG. 1. *Zoospores killed with osmic acid and stained with cotton blue.*

FIG. 2. *Sporangia; a to d—various stages in formation and liberation of zoospores, e—abnormal vesicular sporangium formed in pea broth.*

FIG. 3. *Chlamydospores.*

FIG. 4. *Oospores.*

tough in tube cultures. The hyphae range from 3 to 6μ in diameter, are moderately branched, and become sparsely septate with age. Submerged hyphae are irregular in outline and in branching.

Sporangia are produced on the aerial mycelium that forms on the surface of infected fruits or stems under very humid conditions. They are also produced abundantly in culture. Sporangiophores are undifferentiated from sterile hyphae. Sporangia are broadly ovoid to pyriform in shape, each having a very prominent papilla (Fig. 2 and Plate II, Figs. 16 and 17). Both terminal and intercalary sporangia are found, the former often breaking off but without retaining a pedicel. From measurements of 100 sporangia produced on oatmeal agar their length was found to range from 20.3 to 63.8μ, and to average 40.8μ; their width varied from 14.5 to 49.3μ and averaged 30.0μ; the mean ratio of length to width was found to be 1.35. The contents of mature sporangia appear fairly homogeneous, but, if they are placed in water, zoospores become differentiated within them and are released within 15 to 20 minutes. Each sporangium produces 20 to 30 zoospores. A few sporangia that had germinated by means of germ tubes were observed, one tube apparently forming for each zoospore that failed to emerge.

At the time of their discharge the zoospores are fusiform in shape, 7.7 to 11.0μ long and 4.4 to 7.2μ wide (Fig. 1 and Plate II, Figs. 18 and 19). Each bears two flagella, differing slightly in length, which are attached at a point on the side of the spore in the vicinity of the nucleus. The zoospores swim about actively for about 30 minutes, then settle down, lose their flagella, and become spherical in shape. In this encysted condition they are approximately 8 to 10μ in diameter. They immediately develop germ tubes, the growth of which is sufficiently rapid to be observed with a microscope. These soon become branched and quite extensive. Their development was observed in Petri dishes on moist cellophane, from which they appeared to derive some nourishment since they produced rather extensive colonies which in a few cases bore new sporangia. Cellophane was used by Haskins (7) for the culture of chytrids.

In cultures one month or more old, spherical chlamydospores with thick brownish walls are found (Fig. 3 and Plate II, Fig. 21). Their diameter was found from 100 measurements to vary from 20.3 to 40.6 μ, and to average 31.2μ. They are mostly intercalary and apparently germinate only by germ tubes.

As yet no oospores have appeared in culture on any of the media used. All attempts to induce oospore formation by special treatments failed. When sterile mycelium was transferred from a decoction of canned peas to sterile water (Leonian's (13) method), only sporangia were formed, some of which were abnormally large and vesicular (Fig. 2*e*). Likewise, only sporangia formed when sterile mycelium was transferred to Petri's mineral solution or to $M/100$ potassium nitrate solution (Tucker's (26) methods). No oospores were formed when local isolants were allowed to grow in contact with the isolant sent from British Columbia. A few oospores were discovered, however,

on the surface of roots of a tomato seedling grown in sterile culture then transferred to a test tube of water into which zoospores had been released. Measurements of 37 oogonia showed a range in diameter from 30.0 to 60.0μ and a mean diameter of 50.3μ. The oospores themselves measured 22.5 to 42.5μ, with a mean diameter of 33.0μ (Fig. 4 and Plate II, Fig. 20). It was impossible to determine at this stage whether the attachment of the antheridia was amphigynous or paragynous.

B. Identification of the Causal Organism

From the above observations it was concluded that the causal organism is *Phytophthora parasitica* Dast. In its morphology it corresponds with that species as originally described by Dastur (5) in 1913 and amended by Ashby (1) in 1928. Because of the large oospores it would be placed in the group *Macrospora* established by Ashby. Tucker (26) states that this species "may be distinguished by its growth on media, papillate sporangia, abundant chlamydospores in culture, amphigynous antheridia and growth on corn meal agar at 35° C." With reference to growth on culture media he further states that ". . . . *P. parasitica* usually produces aerial mycelium in profusion. The growth in Petri plates is seldom radiate and appressed . . . . , but tufted and irregular with more or less aerial mycelium." The tufted appearance was especially marked in the present study in colonies on corn meal agar. Also according to Tucker "the occurrence of oogonia and oospores in culture is uncertain. Isolations seldom produce them very promptly; they may appear only after several months or, frequently, not at all." If Leonian's (13) key for identification were followed, this fungus would be identified as *P. palmivora* Butl., since he includes *P. parasitica* with other species in this one species.

Cultural Studies

A. Media

This species of *Phytophthora* grows well on a variety of culture media, including potato dextrose agar, malt agar, and corn meal agar. Of the media tried, unfiltered oatmeal agar supported the most rapid and luxuriant growth of mycelium with abundant sporangia. A suitable liquid culture medium was made up according to the following formula: Bacto peptone, 2.0 gm.; dihydrogen potassium phosphate, 0.5 gm.; magnesium sulphate, 0.5 gm.; succinic acid, 0.2 gm.; dextrose, 5.0 gm.; distilled water, 1000 cc. This is a modification of Leonian's (13) medium, with Bacto peptone substituted for proteose peptone. The fungus does not fruit on this medium unless the colonies reach the surface. Its growth was accelerated slightly by the addition of a trace of Vitamin B_1 in the form of thiamin hydrochloride. Growth was greatly stimulated when the roots of tomato seedlings were added to the medium before autoclaving it. The root of a week-old seedling added to 100 cc. of medium brought about a marked increase in growth. It is not known whether the roots provided a nutrient substance or a growth promoting substance.

B. Effect of Temperature on Growth Rate

On Oatmeal Agar

The effect of temperature on the growth rate of *P. parasitica* was first studied using unfiltered oatmeal agar as the medium. The following temperature range was obtained by using refrigerators, Wisconsin temperature tanks, and incubator ovens: 2°, 5°, 8°, 12°, 16°, 21°, 30°, 32.5°, 37.5°, and 40° C. Three Petri dish cultures were grown at each temperature, and dishes of water were kept in the ovens at 35° to 40° to check the drying of the medium. Uniform disks of inoculum were cut from the margin of a vigorously growing colony on oatmeal agar in a Petri plate by means of a platinum "biscuit cutter". The individual disks were then transferred to the centres of the plates. The outlines of the colonies were marked with India ink on the bottoms of the plates at approximately 12-hour intervals until the colonies had reached the margins of the dishes.

No growth resulted at 2°, 5°, and 8°, or at 35°, 37.5°, and 40° C. After 146 hours one plate from each of these temperatures was brought to 26° and kept there until the end of the experiment. The cultures from the lower temperature group survived and developed, but those from the higher temperatures were dead. The growth curve obtained for the cultures at the remaining temperatures show the minimum temperature for growth on this medium to be about 12° and the maximum temperature about 32.5° C. The greatest amount of growth under these conditions is made over the range from 26° to 32.5° C.

On Potato Dextrose Agar

The experiment was repeated over the same temperature range using as the medium potato dextrose agar. Unfortunately the cultures at the temperatures from 12° to 21° were destroyed through accidental flooding in the temperature tanks. The fungus makes much slower growth on this medium and the colonies are more submerged and irregular in outline. The best growth was made over the same range as before with the largest colonies at 26° C.

On Corn Meal Agar

The experiment was repeated at a later date using filtered corn meal agar as the medium. The temperatures used this time were 8°, 12°, 15°, 18°, 25°, 30°, 32°, 35°, 37.5°, and 40° C. After 168 hours at these temperatures all cultures were brought to room temperature (approximately 20° C.) and kept there for 92 hours longer.

The diameters of the colonies produced by the fungus on these three media at the various temperatures are shown in Fig. 5. The cardinal points obtained for corn meal agar do not closely agree with those found for growth on the other media. The minimum temperature was the same, although much better growth was made at 12° on the corn meal agar. Fairly good growth was obtained at 35° where none resulted before. Even at 37.5° the

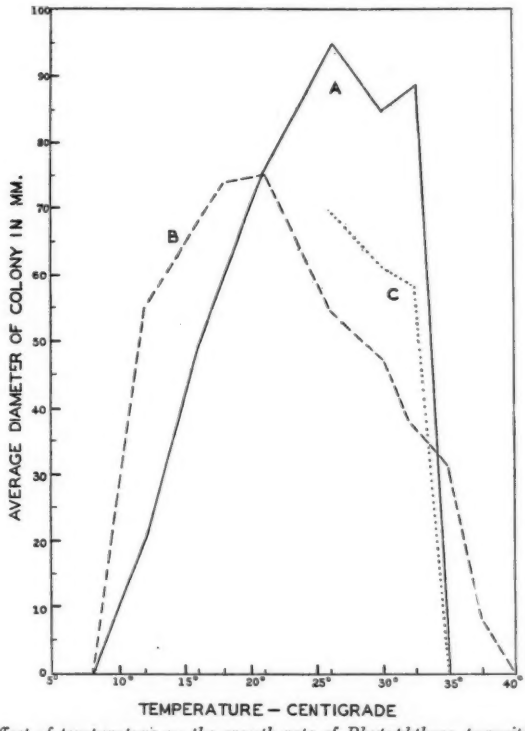


FIG. 5. *Effect of temperature on the growth rate of Phytophthora parasitica on different media. Diameters of colonies produced at the various temperatures by the end of the test periods.*
A. Oatmeal agar, 88 hours.
B. Corn meal agar, 168 hours.
C. Potato dextrose agar, 260 hours.

fungus developed for a few days before it was killed. The temperature range that is optimum for growth on corn meal agar (18° to 21°) is lower than that for growth on oatmeal and potato dextrose agars (26° to 32°). The variation found here in the cardinal points on different media illustrates the necessity for caution in interpreting the effect of the temperature factor on disease incidence in terms of its effect on the vegetative development in culture.

The type of growth produced on corn meal agar changed between 21° and 25°. At 21° and below aerial mycelium was abundant, erect strands of hyphae reaching the lids of the dishes. At 25° and above growth was closer to the surface of the medium and more compact. The margins of the colonies also became more irregular with increasing temperature. The difference in growth habit at this point may be due, in part at least, to the difference in the relative humidity of the atmosphere, since the lower series was kept in tanks with the temperature controlled by flowing water, while the higher series was kept in incubator ovens.

The reactions of the cultures when returned to room temperature are interesting. The cultures from 37.5° and 40° did not grow at all at this temperature, while the cultures that had been kept at 8° developed rapidly. All the other cultures continued to grow, although not all at the same rate. Those that had previously grown fastest at 18° to 25° and covered the greatest area now spread at a slower rate than those (from higher or lower temperatures) that had covered less area. This shows that temperature and not drying of the medium was responsible for the slower growth rates at temperatures above the optimum under these conditions.

C. Effect of Atmospheric Humidity on the Growth of the Fungus in Culture

The effect of the relative humidity of the atmosphere on the growth of *P. parasitica* in culture was studied, following a procedure outlined by Hopp (10). The relative humidity within the confined space of Petri dishes was controlled by using different concentrations of sulphuric acid. Eight cubic centimetres of oatmeal agar were poured into each of 24 75-mm. Petri dishes. Each plate was inoculated as before by inoculum-disk method, then placed within a sterile 100 cc. Petri dish. The lid of the smaller dish was removed, and 15 cc. of sulphuric acid of the required concentration was poured into the outer dish. The concentration of acid required to maintain a given relative humidity in a closed atmosphere over it was found from Hopp's graph. Solutions were prepared that produced atmospheres with initial relative humidities ranging from 0 to 100% with 10% intervals. Plates were prepared in duplicate, and one pair with no solution was used as a control. All plates were incubated at 30° C. for 66 hours, then the diameter of the colony in each was measured.

The size of colony produced in each atmosphere is shown in Table I. No aerial growth was produced at any relative humidity lower than 80%. Below that humidity submerged colonies were formed inconsistently, ranging in size from 10 to 35 mm. These evidently represented the amount of growth possible on the amount of moisture supplied by the medium. The agar at the lower humidities soon dried and cracked. Above 80% relative humidity the colony size increased with increasing humidity to a diameter of 61 mm. at saturation. The check plates produced colonies of a size between those formed at 90 and 100% saturation. Moisture, apparently supplied by the freshly poured agar, condensed on the lids of these dishes, and this is probably the condition in Petri dish cultures during the first few days. The relative humidity later will depend upon the temperature that determines the rate of evaporation. Atmospheric humidity was probably one factor that determined the rate and amount of aerial growth found at different temperatures in the preceding experiment. This factor may also influence the development of the fungus in nature, and explain in part the restriction of lesions to the region of the stem near the ground level.

TABLE I

RELATION BETWEEN ATMOSPHERIC HUMIDITY AND THE GROWTH OF *P. parasitica* IN CULTURE

Conc'n of H_2SO_4, %	Relative humidity, %	Diameter of colony, mm.*		
		Plate 1	Plate 2	Average
95.0	0	35.0	—	35.0
67.0	10	22.0	24.0	23.0
59.0	20	27.0	23.0	25.0
53.5	30	17.0	10.0	13.5
49.0	40	18.0	—	18.0
43.0	50	15.0	—	15.0
38.0	60	33.0	—	33.0
34.0	70	35.0	x	35.0
27.5	80	37.0	41.0	39.0
19.5	90	46.0	49.0	47.5
Water	100	63.0	59.0	61.0
—	Check	55.0	62.0	58.5

* *After 66 hours at 30° C.*
x = *Contamination.*

D. Effect of Acidity of Medium on Growth Rate

In the study of the relation between the pH of the medium and the growth rate of the fungus in culture the liquid medium previously described was used. A preliminary series of solutions was made up with this to determine the amount of 1/10 *N* hydrochloric acid or 1/10 *N* sodium hydroxide required to adjust them to desired pH concentrations. In the solutions actually used for cultures, 50 cc. of water with the nutrient salts were put into each 125 cc. Erlenmeyer flask, while the acid or base was run into a test tube then diluted to 25 cc. with distilled water. Flasks and tubes were plugged and autoclaved separately, then the contents of the tubes were added to the flasks immediately before inoculation. The inoculum disk method of inoculation was used as before, care being taken that the disks settled to the bottom of the flasks so that all growth was submerged. Four flasks were prepared for each of the pH concentrations tested. After inoculation they were kept in an incubator for seven days. The four corresponding cultures were then filtered through a previously weighed filter paper; this was air-dried for 24 hours, then weighed again. The difference between these weights was taken as the dry weight of the mycelium produced. The pH of the filtrate was determined electrometrically. The experiment was repeated over approximately the same pH range.

The original and final pH of the cultures and the dry weights of the mycelium produced in them are shown in Table II. The fungus exhibited a wide range of tolerance with respect to the reaction of its substrate. Growth was possible in the acid range at pH 3.5 and in the alkaline range at pH 9.5. Maximum growth under the conditions of the experiment was attained at pH 5.0. The reaction of the medium was not shifted appreciably except in the alkaline

TABLE II

EFFECT OF pH OF MEDIUM ON GROWTH RATE OF *P. parasitica* IN LIQUID CULTURES

Original pH	Final pH		Dry weight of mycelium, mg.	
	1st test	2nd test	1st test	2nd test
3.0	3.02	—	0.0	—
3.5	3.48	3.55	60.0	50.2
4.0	3.75	3.80	66.0	57.7
5.0	4.64	4.60	124.3	147.8
6.0	5.68	5.48	97.4	128.9
7.0	6.70	7.00	112.6	77.3
8.0	7.85	8.10	76.2	69.2
9.0	8.12	8.25	83.4	52.7
9.5	—	8.35	—	48.9
10.0	8.70	8.70	Trace	0.0

range above pH 8.0. This is due to the instability of the medium and is not caused by the growth of the fungus, since the shift occurred in the cultures started at pH 10.0 where no growth ensued. A slight precipitation was observed in those cultures whose reaction shifted markedly. The slight shifts in the other cultures was usually in the direction of increased acidity.

Epidemiology

A. Atmospheric Environment

Temperature and Relative Humidity

The intensity of the disease caused by this pathogen is influenced markedly by atmospheric conditions. While it was not feasible to carry out experiments under controlled conditions of atmospheric temperature and humidity, observations were made in the course of experiments involving thousands of seedlings grown under varying conditions. From these and from observations made whenever natural epidemics occurred in commercial greenhouses it was concluded that maximum disease incidence was correlated with high air temperatures and high relative humidity. The rate of damping-off of seedlings was greatest on hot, humid days. Natural infection of mature plants occurred only in the months of June, July, August, and September, even where the soil was heavily infested. Temperature and humidity appear, therefore, to be the limiting factors which, directly or indirectly, restrict the disease almost exclusively to plants grown under glass.

B. Soil Environment

Temperature and Moisture

The influence of soil temperature and soil moisture on disease incidence in tomato seedlings was investigated by using the Wisconsin soil temperature tanks in the University of Toronto greenhouses. The air temperature was kept at about 21° C. and the relative humidity varied from about 85 to 90%.

The soil used was a mixture of one-third each of loam, sand, and leaf mould. This was steam sterilized, then infested by adding cultures of *Phytophthora parasitica* growing on a corn meal and sand mixture. Sixteen 500 cc. Erlenmeyer flasks of inoculum were used for about five cubic feet of soil. After thorough mixing, one portion of the soil was adjusted to each of three moisture levels. The first lot was left too dry for good development of tomato seedlings; the second was given a moisture content that was approximately optimum for the host; the third was made too wet for the best development of the host. These three moisture levels will be referred to as "low", "optimum", and "high". Their moisture contents, determined at the end of the experiment from the top two inches of soil, were found to be respectively 34.0, 49.0, and 71.5% of the moisture-holding capacity of this soil.

Each of the soil cans was prepared for planting in the following way. First a 3-in. layer of sterilized sand was put in the bottom for drainage. On top of this an inverted 2-in. flower pot was placed with a glass tube leading from it up to an inch or more above the infested soil which was filled in next. Water was added as required through the glass tube and the pot acted as a reservoir to permit uniform distribution of moisture through the soil and to avoid saturation of the surface layer. The weight of each can was determined at the beginning of the experiment, and the required moisture content was maintained by adding water to bring each can back to its original weight. The need for additional water varied with the temperature. Cans at the higher temperatures were checked every second day, while those at the lower temperatures were checked once a week.

Duplicate cans were prepared in this way at each of the three moisture levels for each of five temperature tanks. The tanks were maintained at five different temperatures: 12°, 17°, 22°, 27°, and 32° C. In each can 150 tomato seeds of the variety Vetomold were planted at a uniform depth. The soil was covered with a layer of non-absorbent cotton to conserve moisture until the seedlings emerged.

Daily records of the number of seedlings damped-off were taken until 36 days after the date of planting. The survivors were then counted and the daily losses totalled. The difference between the total number of seedlings that emerged and the number of seeds that were planted was taken as the amount of pre-emergence blight. The total killing (pre-emergence plus post-emergence) was then expressed as a percentage of the number of seeds planted.

A check series was planted at the same time to ascertain the effect of soil temperature and soil moisture on the germination of tomato seeds in the absence of the pathogen. One check can filled with sterilized soil with optimum moisture content was kept in each temperature tank. In addition, one can of sterilized soil at each moisture level was kept at room temperature (about 21° C.) for the duration of the experiment.

The results for the control series, which are presented in Table III, were not used in computing the amounts of pre-emergence killing in the cans of infested soil. The 26% germination obtained at 12° C. is evidently exceptional,

TABLE III

EFFECT OF VARIOUS TEMPERATURES AND MOISTURE CONTENTS OF STERILIZED SOIL ON THE GERMINATION OF TOMATO SEEDS

Temperature, °C.	Moisture	No. of seeds germinated	Germination, %
12	Optimum	39	26.0
17	Optimum	128	85.3
22	Optimum	143	95.3
27	Optimum	150	100.0
32	Optimum	118	78.6
±21	Low	140	93.3
±21	Optimum	146	97.3
±21	High	134	89.3

since 85% was obtained in the corresponding cans of infested soil. The same discrepancy was found, to a lesser degree, in the other cases. A larger number of replicates at each combination of temperature and moisture would have to be used to give a reliable basis for comparison, but the equipment available would not accommodate them. While assuming 100% germination in the analysis of the results may alter slightly the absolute amount of total killing and the relation between pre- and postemergence killing, this would not distort appreciably the effect of any soil temperature or soil moisture on disease incidence.

TABLE IV

EFFECT OF SOIL MOISTURE AND SOIL TEMPERATURE ON THE AMOUNT OF DAMPING-OFF OF SEEDLINGS DUE TO *P. parasitica*

Soil temperature, °C.	Soil moisture	Survivors, 36 days	Emergence		Damping-off			
			Total	%	Pre-emergence	Post-emergence	Total	%
12	Low	243	245	81.7	55	2	57	19.0
	Opt.	251	255	85.0	45	4	49	16.3
	High	196	234	78.0	66	38	104	34.7
17	Low	152	269	89.7	31	117	148	49.3
	Opt.	113	254	84.7	46	141	187	62.3
	High	27	225	75.0	75	198	273	91.0
22	Low	137	255	85.0	45	118	163	54.3
	Opt.	104	217	72.3	83	113	196	65.3
	High	21	176	58.7	124	155	279	93.0
27	Low	174	261	87.0	39	87	126	42.0
	Opt.	131	211	70.3	89	80	169	56.3
	High	72	300	100.0	0	228	228	76.0
32	Low	205	272	90.7	28	67	95	31.7
	Opt.	146	265	88.3	35	119	154	51.3
	High	79	256	85.3	44	177	221	73.7

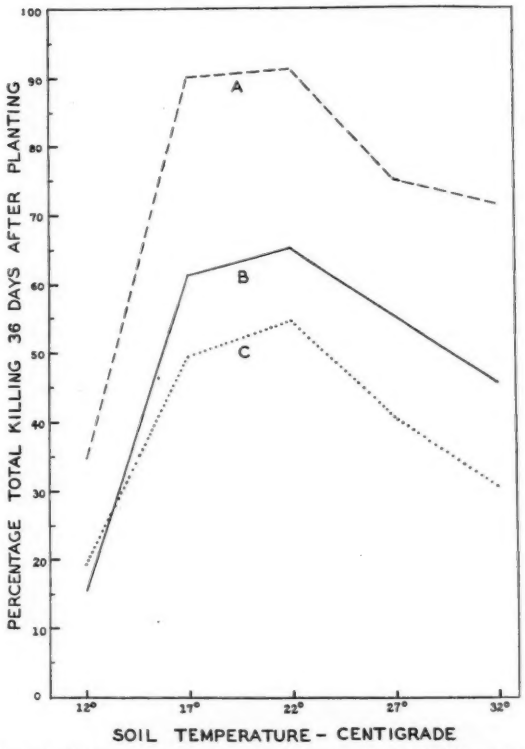


FIG. 6. *Effect of soil temperature and soil moisture on the total killing of tomato seedlings by Phytophthora parasitica in 36 days after planting.*

A. High moisture.

B. Optimum moisture.

C. Low moisture.

The final results of the experiment are shown in Table IV and Fig. 6. The relation between disease incidence and soil moisture is apparent, the amount of disease varying directly with the moisture content of the soil. A broad temperature range from about 15° to 27° C. is favourable for disease, while maximum disease occurs at 22° C. The steepest decline in disease incidence occurs between 17° and 12°. The lack of separation of the curves for low and optimum moisture at 12° may be attributed to the fact that condensation of atmospheric moisture on the sides of the cans and on the soil surface at this temperature made it impossible to keep the low moisture cans at their original moisture content.

On comparing the effect of temperature on disease with its effect on the growth of the causal organism in culture, it is found that the temperature range 15° to 27° which is most favourable for disease also permits good vegetative development of the fungus in culture. Thus the temperature factor

apparently influences the degree of disease incidence largely through its direct effect on the pathogen. That temperature may also favour disease by predisposing the host to attack is suggested by the fact that maximum disease occurs at a point below 27° C. which is the most favourable temperature for the development of the host. At temperatures below the optimum range for disease, the host takes longer to emerge from the soil and pre-emergence blight is greater, in spite of the fact that the pathogen is less active, due to the longer exposure to attack at this stage. The decrease in postemergence killing at low temperatures is apparently due to a depressing effect of the temperature on the development of the pathogen.

C. Factors Influencing the Establishment and the Activity of the Pathogen in the Soil

Establishment in the Soil

Effect of Soil Type

In the following experiment the ease of establishment of the pathogen in two different types of soil was compared. The soils used were an acid, marsh soil, or "muck", and a neutral garden loam. The muck soil was taken from a swamp that is flooded most of the year. Its pH was found to be 6.6, while the loam had a pH of 7.0. One lot of each soil was steam sterilized but this treatment did not change the reaction. One flat was then filled with each of the soil lots—sterile and non-sterile muck, and sterile and non-sterile loam. Each flat was then infested by adding one culture of *P. parasitica* growing on corn meal and sand in a 300. cc. Erlenmeyer flask. After thoroughly mixing the inoculum into it, the soil was left for 10 days before planting. Then 500 seeds were planted at a uniform depth in each flat, as well as in a series of non-infested check flats of each soil lot.

The check series showed no significant difference in the amount of germination in the loam and muck, either sterile or non-sterile. Since the germination was uniformly high in the checks, pre-emergence killing was estimated on the basis of the number of seeds planted. The results of this experiment are recorded in Table V. The pathogen was evidently more successful in estab-

TABLE V

Relative amounts of disease in infested sterile and non-sterile loam and marsh soil

Soil type	Survivors, 30 days	Emergence		Damping-off			
		Total	%	Pre-emergence	Post-emergence	Total	%
Non-sterile muck	203	370	74.0	130	167	297	59.4
Sterilized muck	198	361	72.2	139	163	302	60.4
Non-sterile loam	174	450	90.0	50	276	326	65.2
Sterile loam	29	354	70.8	146	325	471	94.2

lishing itself in some of the soil lots than in others, according to the trends shown in disease incidence. The amount of total killing was approximately the same in the infested sterile and the non-sterile marsh soil, but it was much greater in the sterile loam than in the non-sterile loam. The amount of pre-emergence killing in non-sterile loam was roughly only one-third of that in the other three lots, though here the postemergence killing was much higher than in the muck soils.

The two soil types differed, presumably, (i) in their microfloral population, as well as (ii) in acidity, and (iii) in physical condition. The differences in ease of establishment of the pathogen in them might, therefore, be attributed to one or more of these features. That the microfloral populations were a factor is shown by a comparison of the disease trends in the sterile and the non-sterile series. The difference in disease incidence in sterile and non-sterile loam is very striking. Pre-emergence killing was 50 in non-sterile loam and 146 in sterile loam, while postemergence killing was 276 and 325, respectively. Thus some factor, which is present in non-sterile loam but not in sterile loam, suppresses temporarily the aggressiveness of the pathogen. This factor is most likely the competitive effect between the pathogen and the other soil organisms present in the non-sterile loam. This evidently did not affect the establishment of the pathogen in non-sterile muck, since the amounts of both pre- and postemergence killing there were practically identical with the amounts in the sterilized muck. The pathogen apparently found the predominant members of the microfloral population of this sample of marsh soil compatible, whereas it had more difficulty in establishing itself in the presence of the microflora of the loam. Whether this difference was due to the specific effect of one organism present in the one soil and not in the other, or whether it is due to a difference in the resultant of the dominant forms in the two cases could only be shown by a study of the soil populations by isolation methods. The slight difference in acidity between the two series was evidently not a factor influencing establishment, since no correlation was found between pH and disease. The physical condition of the soil was apparently a factor, since disease incidence was much higher in the sterile loam than in the sterile muck when the biological factor, except for saprophytic forms that come in after sterilization, is ruled out. This physical factor seems to have more effect on the postemergence killing than on the pre-emergence, since twice as much postemergence killing occurred in the sterile loam as in the sterile muck whereas the amounts of pre-emergence killing were roughly the same. One physical character of the soils that could conceivably influence disease incidence in this way is the water-holding capacity. The marsh soil absorbed water very readily and remained porous, while the loam was easily saturated and remained soggy for some time after watering. The pathogen would likely have an equal chance in the two cases of attacking seedlings before they emerged, but if it depended on free water to release zoospores for attack on aerial portions of the seedlings, it would be more successful in the loam.

Effect of Mixing Infested Soil with Various Proportions of Sterile and Non-sterile Soil

In order to study further the competitive effects influencing the establishment of the pathogen in the soil, the following experiment was carried out. Using the infested soil which had been inoculated four months previously and used subsequently in the soil temperature experiment, a dilution series was made up. In the first half of the series the infested soil was mixed with various amounts of freshly mixed greenhouse soil composed of one-third loam, one-third leaf mould, and one-third sand. In the other half of the series the infested soil was mixed with the same amounts of steam sterilized soil of the same composition. One flat was then made up with each of the following proportions of infested and non-infested soil.

Flat No.	Proportions		Flat No.	Proportions	
	Infested	Non-sterile		Infested	Sterile
1	All	None			
2	7/8	1/8	8	7/8	1/8
3	3/4	1/4	9	3/4	1/4
4	1/2	1/2	10	1/2	1/2
5	1/4	3/4	11	1/4	3/4
6	1/8	7/8	12	1/8	7/8
7	None	All (check)	13	None	All (check)

In the first test, all flats were planted May 22 with 500 seeds each and covered to a uniform depth with the appropriate soil mixture. Daily records of the number of seedlings killed were kept for 30 days after planting. The survivors were then removed and counted, and the amount of pre-emergence killing was computed by subtracting the total number of seedlings that emerged in each flat from the number that germinated in the corresponding sterile or non-sterile check. The total killing was also expressed as a percentage of the germination in the appropriate check.

The second planting was made on June 24 in the same flats. The soil was loosened by stirring, then an equal part was removed from each and used for covering the seeds after planting. Records were kept for another 30 days, then the results were computed as before.

The results obtained from both plantings are shown in Table VI. The amount of total killing in the flats in the first planting varied with the relative amount of infested soil in the various mixtures. Thus maximum killing resulted in the flat of undiluted infested soil (Flat 1), and the disease incidence in the other corresponding pairs decreased with decreasing amounts of infested soil. Flat 4 was abnormal due to accidental flooding two days after planting. That flat remained much wetter than the others for a week or more though not watered further. Pre-emergence killing was exceptionally high in this case. Postemergence killing was also high for a few days but fell to a level

TABLE VI

AMOUNTS OF DISEASE CAUSED BY *Phytophthora parasitica* IN SEEDLINGS PLANTED IN INFESTED SOIL MIXED WITH VARIOUS PROPORTIONS OF STERILE AND NON-STERILE SOIL

Flat No.	Composition of soil	First planting						Second planting					
		Survivors, 30 days	Total emergence	Damping-off				Survivors, 30 days	Total emergence	Damping-off			
				Pre-emergence	Post-emergence	Total	%*			Pre-emergence	Post-emergence	Total	%*
1	All infested	21	418	52	397	447	95.5	129	431	46	302	348	72.9
2	7/8 infested 1/8 non-sterile	17	425	25	408	433	96.2	66	422	23	356	379	85.1
3	3/4 infested 1/4 non-sterile	33	423	27	390	417	92.7	143	418	27	275	302	67.8
4	1/2 infested 1/2 non-sterile	167	336	114	169	283	62.9	121	435	10	314	324	72.8
5	1/4 infested 3/4 non-sterile	83	436	14	353	367	81.6	110	419	26	309	335	75.3
6	1/8 infested 7/8 non-sterile	125	448	2	323	325	72.0	166	385	60	219	279	62.6
7	All non-sterile (check)	448	450	0	2	2	0.4	445	445	0	0	0	0.0
8	7/8 infested 1/8 sterile	25	433	35	408	443	94.6	62	421	56	359	415	87.0
9	3/4 infested 1/4 sterile	29	456	12	427	439	93.8	47	402	75	355	430	90.1
10	1/2 infested 1/2 sterile	27	452	16	425	441	94.2	61	358	119	297	416	87.2
11	1/4 infested 3/4 sterile	59	475	−7	416	409	87.3	116	378	99	262	361	75.6
12	1/8 infested 7/8 sterile	113	468	0	355	355	75.8	148	376	101	228	329	68.9
13	All sterile (check)	468	468	0	0	0	0.0	477	477	0	0	0	0.0

* *Percentage of germination in corresponding check.*

lower than that found in any of the other flats. The seedlings in this flat remained dwarfed and yellowish in colour as a result of the excess moisture. This hardened condition of the plants may account for the early decline in disease incidence there. When corresponding pairs of the normal flats are compared, it is found that the amount of total killing is invariably greater in the case of the flat diluted with sterile soil than in that diluted with non-sterile. This difference became more apparent as time went on, suggesting that the pathogen was becoming established with greater success in the sterile soil than in the non-sterile.

The results of the second planting show a further reduction in disease in the non-sterile as compared with the sterile mixtures. This confirms the tendency shown in the first planting for the pathogen to be suppressed in its aggressiveness, apparently by some biological factor present in the non-sterile soil. Whereas the results in the sterile series appears like a dilution effect with increasing admixtures of sterile soil, the non-sterile series exhibits a definite suppressive effect on the ability of the pathogen to cause disease. Both pre-emergence and postemergence killing were greater in the sterile mixtures than in the non-sterile mixtures. In the first planting the amount of pre-emergence blight was roughly the same in all normal cases. While in the second planting the non-sterile mixtures still showed the same amount, in the sterile mixtures it was doubled. Apparently the pathogen had become better established there, probably due to the initial absence of competition.

There was considerably less killing during the second test in the flat of undiluted infested soil (Flat 1). The reason for this is not clear. It suggests the staling effect found in old cultures of fungi. It might be the result of competition with saprophytic micro-organisms coming into the once sterile soil. It is also possible that exhaustion of nutrient salts, especially nitrates, resulting from the repeated plantings of tomato seedlings in the soil, increased the resistance of the host to attack. This would be overcome in the other flats which received a portion of fresh soil.

Decline in Degree of Soil Infestation with Time

The degree of infestation was determined for the third time for the most dilute mixtures of infested and non-infested soil—i.e., the 1/8 : 7/8 mixtures in Flats 6 and 12. These were replanted as before on August 13 and the course of the disease was followed for another 30-day period.

The data for these flats in all three trials are brought together in Table VII. From these it is evident that the disease rate falls off appreciably as time

TABLE VII

DECLINE IN THE DEGREE OF INFESTATION OF SOIL BY *P. parasitica* AS SHOWN BY THE AMOUNTS OF KILLING OF TOMATO SEEDLINGS PLANTED IN IT

Composition of soil	Date of planting	Survivors, 30 days	Emergence		Damping-off			
			Total	%	Pre-emergence	Post-emergence	Total	%*
No. 6	May 22	125	448	89.6	2	323	325	72.0
1/8 infested,	June 24	166	385	77.0	60	219	279	62.6
7/8 non-sterile	Aug. 13	264	383	76.6	62	119	181	40.7
No. 12	May 22	113	468	93.6	0	355	355	75.8
1/8 infested,	June 24	148	376	75.2	101	228	329	68.9
7/8 sterile	Aug. 13	257	399	79.8	78	142	220	46.1

* *Percentage of germination in corresponding check.*

goes on. In every case the mixture with sterile soil shows more disease than the mixture with non-sterile soil. The differences are slight, it is true, but they are consistent. The amount of pre-emergence blight in both cases was practically nil in the first trial immediately after mixing the soils, but increased in the later tests, especially in the sterile mixture. This again suggests that the pathogen had not become well established in the soil in the first trial, and that it became more generally distributed, according to the later trials, in the sterile than in the non-sterile mixture.

Field Plot Experiments

The establishment of *P. parasitica* in natural soil was also studied under field conditions. A series of plots were inoculated by adding corn meal and sand cultures of the pathogen. The soil in half of these plots had been previously removed, steam sterilized and replaced to temporarily remove the biological factor. The activity of the parasite was shown by observing the trends in disease incidence in tomato seedlings over two 30-day test periods. The field experiments confirmed the greenhouse tests in demonstrating a retarding factor that is present in non-sterile soils but not present in sterilized soils. Competition with the fungal and bacterial population of non-sterile soils presumably accounts for such an effect. Other organisms may compete for the substrate required by the pathogen, or they may release into the soil by-products that retard its development. No attempt was made to isolate organisms with such antibiotic tendencies.

The Invasion of Non-infested Soils by the Pathogen

Spread from a Single Centre of Infection

The successful infestation of soil from a single centre of infection originating from a minute amount of inoculum was demonstrated by a simple experiment. Twenty apparently healthy tomato seedlings that had been growing for 30 days in infested soil were transplanted into 5-in. pots, 10 of which contained sterilized greenhouse soil, the other 10 non-sterile soil. As much of the original soil as possible was shaken from the roots before replanting. Two weeks later, four pots were selected from each lot and the plants, which had been killed by that time, were carefully pulled out without disturbing the soil. In each pot 16 seeds were next inserted into the soil, by means of forceps, in the arrangement shown in Fig. 7—i.e., two in the position of the original plant, four in the inner ring about this point, and 10 in the outer ring. For three weeks after their planting the dead seedlings were removed daily and their position was noted. Those surviving were left for a further three weeks before a final count was made.

Most of the seeds planted in the exact position of the original plant did not germinate, and the few seedlings that did come up at that point were soon killed. Seedlings in the other rings were killed progressively outwards from the centre. Fig. 7 represents a typical pot from each lot. Three weeks after planting the situation was as follows: in non-sterile soil—36 alive, 15 damped-

off; in sterile soil—32 alive, 21 damped-off. Three weeks later only two seedlings were alive in the non-sterile soil, while six survived in the sterile. The number of individuals used was small but the experiment does indicate the ability of the pathogen to establish itself and spread in either sterile or non-sterile soil to a degree that makes it a potentially destructive contaminant in greenhouse soil, whether introduced en masse in a soil lot or in minute quantities with young plants or on tools or workers' boots.

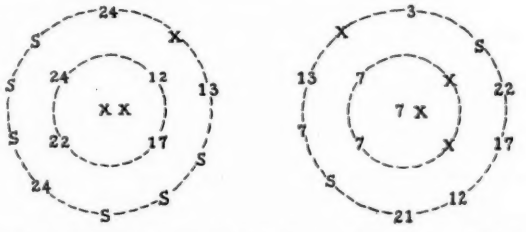


FIG. 7. *Diagram showing the spread of the disease from centres of infection; left: non-sterile soil, right: sterile soil. Numerals mark position of dead seedlings and represent number of days between planting and killing.*
x:— seedling failed to emerge. *s:— living seedling.*

It is interesting to note the behaviour of the original plants in these pots. Although they appeared healthy when transplanted into the pots, within a week every plant showed definite symptoms of infection, and by the end of two weeks all but two had succumbed. One of these was in the sterile soil, the other in the non-sterile. If they had been left undisturbed in the original infested soil very few of them would have been killed at that stage. Thus transplanting appeared either to weaken the resistance of the host to fresh infection by the pathogen or to stimulate the development of incipient or abortive infection.

Linear Spread in Flats of Soil

Experiment I

To study further the relative rates of invasion of sterile and non-sterile soils by *P. parasitica*, the following experiment was devised. A very thin partition of wood was placed across the centre of each of two flats. One-half of each flat was then filled with soil that had been infested four months previously and used in subsequent experiments. The other half of the first flat *(A)* was then filled with a non-sterile mixture of loam, leaf mould, and sand. The other half of the other flat *(B)* was filled with the same soil mixture after it had been steam sterilized. Ten rows, two inches apart, were marked across each flat by means of a wooden marker, so that five rows were across each type of soil. Fifty tomato seeds were planted in each row on June 4 and covered with as little disturbance of the soil as possible. The partitions were then removed and the infested and non-infested soils came in contact with one another. Records of the number of seedlings killed each day were kept for 30 days. At the end of that time the survivors were counted

and the number in each row that failed to emerge was determined. The surviving seedlings were carefully removed, fresh rows were marked in the same places as before, and a second planting was made at once (July 4) without disturbing the soil. Records of damping-off were taken for a second 30-day period.

The advance of the pathogen into the non-infested soils may be traced by following the progress of the disease as recorded in Table VIII. The results at the end of each period are presented in the form of a histogram in Fig. 8. During the first test the spread of the pathogen into the non-sterile

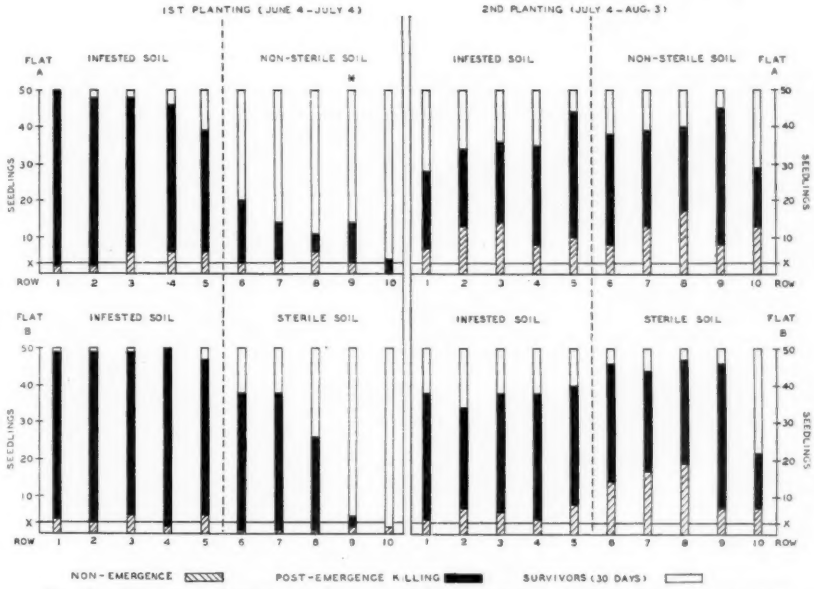


FIG. 8. *Histogram showing the advance made by P. parasitica from infested soil into sterile and non-sterile soils by the end of 30 days and 60 days, as indicated by the numbers of seedlings killed in each row. Horizontal line at x indicates the average amount of non-emergence obtained for this seed lot when planted in non-infested soil.*

* *Surface contamination.*

soil in Flat *A* was slow and irregular. A spot infection that occurred in Row 9 was apparently the result of surface contamination, since the rows between it and the infested soil escaped disease until later. The advance of the pathogen into the sterile soil in Flat *B* was more rapid and uniform, progressing row by row, and reaching Row 9 by the 30th day.

During the second trial the pathogen became well established throughout the non-sterile soil in Flat *A*. It seemed to spread from two centres, from the line of contact with the infested soil and from the contaminated spot mentioned previously. The sterile soil in Flat *B* became heavily and uniformly infested. About 90% mortality resulted in all rows there except Row 10

TABLE VIII

INVASION OF STERILE AND NON-STERILE SOILS OF *P. parasitica* AS SHOWN BY THE PROGRESSIVE DAMPING-OFF OF SEEDLINGS
DAILY LOSSES UP TO 30 DAYS FROM PLANTING

Soil	Row	Number of days from planting																							
		7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
		First planting																							
Flat *A*—Non-sterile Infested	1	—	0	0	0	2	—	0	1	0	0	1	2	—	6	8	17	7	1	0	—	—	3	0	0
	2	—	3	13	3	1	—	1	0	0	0	0	1	—	8	3	6	5	1	0	—	—	1	0	0
	3	—	1	15	2	3	—	0	0	1	0	0	1	—	9	1	5	3	0	1	—	—	0	0	0
	4	—	1	5	3	6	—	1	2	1	0	0	5	—	12	4	0	0	0	0	—	—	0	0	0
	5	—	0	0	1	1	—	4	1	2	0	2	8	—	15	0	0	2	1	0	—	—	1	0	0
Non-infested	6	—	0	2	1	0	—	0	0	0	0	0	0	—	1	0	4	5	0	1	—	—	2	0	1
	7	—	0	2	0	0	—	0	0	0	0	0	0	—	0	0	0	0	0	0	—	—	0	3	5
	8	—	0	0	0	0	—	0	0	0	0	0	0	—	0	0	0	0	0	2	—	—	1	1	1
	9	—	0	0	0	0	—	0	1	0	0	0	0	—	0	1	0	4	0	0	—	—	0	1	4
	10	—	0	0	0	0	—	0	0	0	0	0	0	—	0	0	0	0	0	0	—	—	0	1	3
Flat *B*—Sterile Infested	1	—	1	19	3	0	—	1	1	0	0	0	1	—	7	3	7	0	1	0	—	—	0	0	1
	2	—	5	27	3	1	—	1	0	1	0	1	0	—	3	3	1	0	0	0	—	—	0	0	0
	3	—	4	21	3	0	—	0	0	0	0	0	2	—	1	1	10	2	0	0	—	—	0	0	0
	4	—	1	13	4	1	—	2	0	0	0	0	3	—	10	6	5	2	0	1	—	—	0	0	0
	5	—	1	19	5	0	—	2	0	0	0	1	2	—	7	2	1	1	0	0	—	—	0	1	0
Non-infested	6	—	0	1	0	0	—	0	0	0	0	0	1	—	11	5	12	4	2	0	—	—	1	0	0
	7	—	0	1	0	0	—	0	0	0	0	0	0	—	4	3	8	11	4	3	—	—	3	0	0
	8	—	0	0	0	0	—	0	0	0	0	0	0	—	0	0	0	2	2	1	—	—	12	4	4
	9	—	0	0	0	0	—	0	0	0	0	0	0	—	0	0	0	0	0	0	—	—	0	0	3
	10	—	0	0	0	0	—	0	0	0	0	0	0	—	0	0	0	0	0	0	—	—	0	0	0

TABLE VIII—*Concluded*

INVASION OF STERILE AND NON-STERILE SOILS BY *P. parasitica* AS SHOWN BY THE PROGRESSIVE DAMPING-OFF OF SEEDLINGS
DAILY LOSSES UP TO 30 DAYS FROM PLANTING—*Concluded*

Soil	Row	Number of days from planting																							
		7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
		Second planting																							
Flat *A*—Non-sterile Infested	1	0	0	2	—	0	1	1	0	0	0	—	1	1	0	0	0	0	—	0	0	1	6	6	2
	2	0	0	0	—	1	1	3	0	1	2	—	2	0	1	0	0	0	—	2	1	2	1	3	1
	3	0	0	0	—	1	6	1	0	0	2	—	2	0	0	0	0	0	—	1	1	4	1	1	2
	4	0	0	0	—	4	4	4	0	1	3	—	3	0	0	0	0	0	—	0	0	1	3	1	3
	5	0	1	2	—	5	12	3	1	2	0	—	2	0	0	1	1	0	—	2	0	1	0	1	0
Non-infested	6	1	0	1	—	8	9	3	1	1	1	—	1	1	1	0	0	0	—	0	0	1	1	0	0
	7	0	2	1	—	5	6	0	0	2	3	—	1	1	1	0	1	0	—	0	1	2	0	0	0
	8	0	0	0	—	3	10	2	1	2	2	—	1	0	0	0	0	0	—	0	0	0	0	1	1
	9	0	0	1	—	5	9	6	3	5	3	—	3	0	0	0	0	0	—	1	0	0	0	1	0
	10	0	0	0	—	0	2	4	0	1	3	—	0	1	1	1	1	0	—	0	0	1	1	0	0
Flat *B*—Sterile Infested	1	0	0	0	—	20	8	2	0	0	0	—	0	0	0	0	0	0	—	0	0	0	1	1	0
	2	0	0	0	—	11	9	1	0	0	1	—	1	0	0	0	0	0	—	0	0	1	2	1	0
	3	0	0	0	—	5	4	1	2	0	2	—	2	0	0	0	1	0	—	2	1	12	1	1	0
	4	0	0	0	—	6	4	3	0	1	1	—	1	0	1	0	0	0	—	0	4	6	1	3	4
	5	0	1	0	—	5	6	2	1	1	2	—	2	2	0	0	0	1	—	1	1	3	3	0	1
Non-infested	6	4	1	2	—	8	5	3	3	0	0	—	2	0	0	1	0	0	—	1	0	0	1	0	0
	7	1	1	0	—	5	7	2	1	3	0	—	2	1	1	0	0	0	—	3	0	0	0	0	0
	8	1	0	1	—	3	10	6	1	0	0	—	0	0	0	0	0	0	—	1	0	1	1	0	3
	9	0	0	0	—	8	11	10	0	1	2	—	3	1	0	0	0	0	—	0	1	1	0	1	0
	10	0	0	0	—	1	3	2	0	0	1	—	4	0	0	0	0	0	—	1	0	0	2	1	0

which tended to be drier, being at the end of the flat. In both cases the amount of total killing was even higher in the recently invaded soil, whether sterile or non-sterile, than in the originally infested soil. The germinability of the seed in non-infested soil was found, from the first plantings in this experiment and the next, to be 94%. Thus any marked increase in non-emergence over three plants per row may be attributed to pre-emergence killing by the pathogen. Pre-emergence blight was evident in the second test in those rows that had been reached by the pathogen by the end of the first test.

Experiment II

The procedure of this experiment was identical with that in Experiment I except that the times of planting were varied in order to find whether the pathogen was able to invade non-infested soils in the absence of its host. For this reason the first planting was delayed until June 13, or nine days after the soils were allowed to come in contact with one another. Also, one month elapsed between the first and second plantings.

The results obtained in this case are shown in Table IX and Fig. 9. In the first trial the pathogen advanced only to the first row in the non-sterile soil in Flat *C*. A spot of infection from contamination also occurred in Row 9. Likewise only the first row in the sterile soil in Flat *D* was affected during this

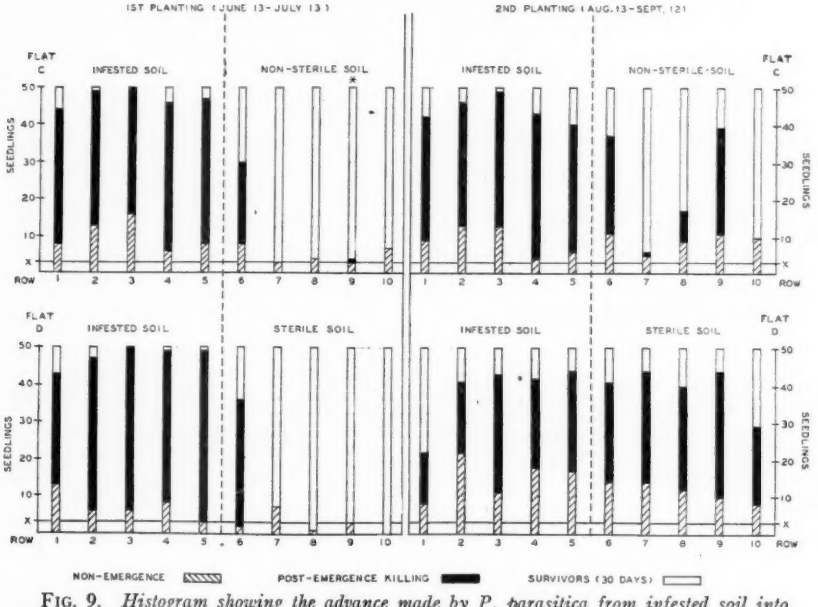


FIG. 9. *Histogram showing the advance made by P. parasitica from infested soil into sterile and non-sterile soils as indicated by the number of seedlings killed in each row. First planting nine days later than in Fig. 8; second planting 30 days after the end of the first test period.*

* *Surface contamination.*

test. Evidently the pathogen did not advance into the non-infested soils until the seedlings had been growing in it for some time, since in both experiments about three weeks elapsed between the first planting and the advance of the disease from the infested half of the flats. This period was not shortened by the 9-day delay in planting in Experiment II. It is difficult to explain why the pathogen did not advance beyond the first row in the non-infested soil in this trial.

Although one month elapsed before these flats were replanted, the disease at first affected only the area it had covered by the end of the first test. It spread progressively thereafter, reaching Row 9 in the non-sterile soil in Flat *C*, and affecting all rows in the sterile soil in Flat *D* by the end of the 30-day test period. The spot infection noted in Row 9 at the end of the first trial increased considerably during the second trial. The amounts of emergence in all rows in the non-infested soils were low after the second planting, but this was evidently caused by some factor other than the pathogen since post-emergence killing did not follow at once but advanced progressively from Row 6.

This experiment indicates an inability of the pathogen to migrate through the soil in the absence of its host. Its advance was not checked in the interim by lack of moisture, since the flats were well watered. Evidently its advance depends upon the presence of living roots of tomato plants. It may not necessarily have to parasitize these roots in order to advance. Abundant mycelium with sporangia has been observed along the surface of roots of seedlings growing in water without any evidence of penetration or breakdown in the host tissues (Plate II, Fig. 16). Also, the presence of roots of seedlings in liquid culture medium was found to stimulate the growth of the fungus. It is quite possible, therefore, that some by-product of the host that is essential for the development of the pathogen is present in the rhizosphere. This by-product may be a growth promoting substance such as auxin. If the pathogen does migrate along living roots, then the delay in its advance into non-infested soil may be accounted for by the time taken for the seedlings to develop root systems which intermingle.

Influence of Various Plant Residues in the Soil on the Aggressiveness of the Pathogen

One of the factors that influence the microbiological balance of a soil is the available organic substrate. Those micro-organisms that can best use the substrate in a given soil will predominate in that soil. If the substrate is changed, the balance will be shifted in favour of other organisms that are better able to use the new substrate. One way of changing the substrate is to add plant residues to the soil. The direction in which the microbiological balance becomes shifted will depend upon the type of decomposition that this plant material undergoes. Certain plants undergo protein degradation and release organic nitrogen, while other plants yield carbohydrates on decomposition. Thus one of two distinct groups of organisms will be favoured by

TABLE IX

INVASION OF STERILE AND NON-STERILE SOILS BY *P. parasitica* AS SHOWN BY THE PROGRESSIVE DAMPING-OFF OF SEEDLINGS
DAILY LOSSES UP TO 30 DAYS FROM PLANTING

Soil	Row	Number of days from planting																						
		8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
		First planting																						
Flat *C*—Non-sterile Infested	1	—	0	—	0	0	0	0	0	0	—	—	2	2	3	11	6	—	3	3	2	3	0	1
	2	—	0	—	0	0	0	1	2	2	—	—	9	7	3	10	2	—	0	0	0	0	0	0
	3	—	4	—	4	1	0	0	0	7	—	—	10	2	4	3	1	—	1	0	0	0	0	1
	4	—	0	—	0	1	3	1	2	2	—	—	14	7	2	3	2	—	2	0	1	0	0	0
	5	—	0	—	0	1	'1	6	4	3	—	—	9	11	1	2	0	—	1	0	0	0	0	0
Non-infested	6	—	0	—	0	0	0	0	0	0	—	—	0	0	0	1	4	—	4	5	4	1	0	3
	7	—	0	—	0	0	0	0	0	0	—	—	0	0	0	0	0	—	0	0	0	0	0	0
	8	—	0	—	0	0	0	0	0	0	—	—	0	0	0	0	0	—	0	0	0	0	0	0
	9	—	0	—	0	0	0	0	0	0	—	—	0	0	0	0	0	—	0	0	0	0	0	1
	10	—	0	—	0	0	0	0	0	0	—	—	0	0	0	0	0	—	0	0	0	0	0	0
Flat *D*—Sterile Infested	1	—	1	—	3	3	2	0	1	3	—	—	4	2	5	4	1	—	0	1	1	0	0	0
	2	—	1	—	1	1	4	1	4	0	—	—	5	5	6	6	3	—	1	0	0	0	1	0
	3	—	1	—	1	1	1	2	2	2	—	—	11	3	3	5	3	—	2	0	0	0	0	0
	4	—	0	—	2	2	3	2	2	2	—	—	3	3	9	4	5	—	0	1	1	0	1	0
	5	—	0	—	0	0	0	0	2	5	—	—	12	5	10	9	1	—	1	1	0	0	0	0
Non-infested	6	—	0	—	0	0	0	0	0	0	—	—	0	0	1	0	4	—	7	7	3	0	4	8
	7	—	0	—	0	0	0	0	0	0	—	—	0	0	0	0	0	—	0	0	0	0	0	0
	8	—	0	—	0	0	0	0	0	0	—	—	0	0	0	0	0	—	0	0	0	0	0	0
	9	—	0	—	0	0	0	0	0	0	—	—	0	0	0	0	0	—	0	0	0	0	0	0
	10	—	0	—	0	0	0	0	0	0	—	—	0	0	0	0	0	—	0	0	0	0	0	0

TABLE IX—*Concluded*

INVASION OF STERILE AND NON-STERILE SOILS BY *P. parasitica* AS SHOWN BY THE PROGRESSIVE DAMPING-OFF OF SEEDLINGS
DAILY LOSSES UP TO 30 DAYS FROM PLANTING—*Concluded*

Soil	Row	Number of days from planting																							
		8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
		Second planting																							
Flat *C*—Non-sterile Infested	1	1	0	2	—	3	0	0	1	2	1	—	—	7	1	0	1	0	—	2	3	4	3	1
	2	3	0	3	—	6	1	2	2	1	0	—	—	7	1	0	1	1	—	1	2	1	0	1
	3	1	1	6	—	13	1	0	0	0	2	—	—	4	3	2	1	0	—	2	0	0	0	0
	4	2	0	4	—	6	4	2	3	2	1	—	—	4	0	2	2	1	—	1	3	0	0	1
	5	1	0	4	—	8	4	4	1	1	3	—	—	4	1	1	0	0	—	0	1	1	0	0
Non-infested	6	3	0	5	—	1	1	1	0	1	0	—	—	4	2	1	2	0	—	3	1	0	0	1
	7	0	0	0	—	0	0	0	1	0	0	—	—	0	0	0	0	0	—	0	0	0	0	0
	8	0	0	0	—	0	0	0	0	0	0	—	—	0	0	1	0	0	—	2	0	4	0	1
	9	0	0	2	—	5	1	0	0	3	1	—	—	4	5	0	4	1	—	0	2	0	0	0
	10	0	0	0	—	0	0	0	0	0	0	—	—	0	0	0	0	0	—	0	0	0	0	0
Flat *D*—Sterile Infested	1	1	0	0	—	2	0	2	0	0	0	—	—	5	1	0	0	1	—	1	0	0	0	1
	2	1	0	0	—	2	0	2	0	1	0	—	—	5	2	3	1	0	—	1	0	0	0	1
	3	0	0	0	—	4	5	1	0	2	2	—	—	16	0	1	0	0	—	0	1	0	0	0
	4	0	0	1	—	2	0	1	0	1	0	—	—	8	2	2	3	1	—	2	0	1	0	0
	5	0	0	0	—	3	2	0	0	1	0	—	—	12	2	2	2	0	—	2	0	1	0	0
Non-infested	6	1	0	0	—	1	1	2	0	2	0	—	—	8	3	1	0	2	—	5	0	0	0	1
	7	1	0	0	—	5	1	2	1	2	0	—	—	7	2	6	2	0	—	1	0	0	0	0
	8	0	0	2	—	7	1	2	1	1	1	—	—	6	2	0	4	1	—	0	0	0	0	0
	9	0	0	0	—	1	1	0	0	0	0	—	—	6	9	2	5	2	—	3	1	4	0	0
	10	0	0	0	—	1	0	0	0	1	0	—	—	5	0	1	1	0	—	1	4	6	1	0

a given plant residue. The group that dominates will tend to suppress the activity of the other group.

To study the effect of plant residues on the activity of *P. parasitica* in the soil the following experiment was carried out. Equal amounts of various plant residues were added to flats of infested non-sterile soil. This soil had been used in previous experiments and the pathogen had become well established. The residues were stems and leaves of fresh corn, soybean, timothy, and tomato plants. This material was run through a meat chopper and about 1500 cc. of each were mixed thoroughly with each flat of soil. The same day 500 tomato seeds were planted in each flat and in one check flat of infested soil. Records of the numbers killed each day were kept for 30 days, then the survivors were removed and counted. A second 1500 cc. portion of fresh residue was added to each flat which was then replanted immediately. On completion of this test, 30 days later, a third test was made without the addition of further residues. This time an inorganic nutrient solution, containing potassium acid phosphate, calcium nitrate, magnesium sulphate, and ammonium nitrate, was supplied to overcome the nitrogen deficiency that was apparent in some of the flats in the previous test.

The results of these tests are presented in Table X. There was little difference in the disease trends at first. In the first test the amount of total killing was slightly lower in the flats that received timothy and tomato residues than the others which were essentially the same as the check. In the second planting the timothy flat showed the greatest amount of disease, while the least killing occurred in the flat with soybean residue. In the third

TABLE X

EFFECT OF VARIOUS PLANT RESIDUES IN THE SOIL ON DISEASE INCIDENCE

Test No.	Residue added	Survivors, 30 days	Emergence		Damping-off			
			Total	%	Pre-emergence	Post-emergence	Total	%
1	Check	67	256	51.2	244	189	433	86.6
	Corn	82	314	62.8	186	232	418	83.6
	Soybean	72	285	57.0	215	213	428	85.6
	Timothy	116	334	66.8	166	218	384	76.8
	Tomato	114	313	62.6	187	199	386	77.2
2	Check	181	448	89.6	52	267	319	63.8
	Corn	200	349	79.8	151	149	300	60.0
	Soybean	269	400	80.0	100	131	231	46.2
	Timothy	115	360	72.0	140	245	385	77.0
	Tomato	193	377	75.4	123	184	307	61.4
3	Check	175	313	62.6	187	138	325	65.0
	Corn	155	244	48.8	256	79	335	67.0
	Soybean	294	369	73.8	131	75	206	41.2
	Timothy	183	290	58.0	210	107	317	63.4
	Tomato	191	301	60.2	199	110	309	61.8

trial the soybean flat showed a definite reduction in both pre- and post-emergence killing. The other flats showed essentially the same degree of disease incidence as the check flat containing no residue. Apparently either the products of the breakdown of the soybean plant or the microbiological activity associated with it retard the aggressiveness of the pathogen. Of the residues used, soybean is the only one that yields carbohydrate and not organic nitrogen on decomposition. It may be that certain carbohydrates favour a group of organisms that suppress the activity of *P. parasitica* in the soil. With abundant organic nitrogen as a substrate, these suppressing organisms may not be dominant but may give way to another group with which the pathogen is compatible. The residue of the normal host of the pathogen does not appreciably favour or suppress its aggressiveness.

Substantiation for this hypothesis was sought in the reaction of the pathogen to the addition of organic nitrogen, in the form of gelatine, and a carbohydrate, dextrose, to infested soils, but the results of these limited tests were inconclusive. Further experiments along this line are desirable.

Host Range and Varietal Resistance

Natural infection has not been observed on any host other than varieties of *Lycopersicon esculentum* Mill. However, artificial inoculations have revealed a wide range of potential hosts, almost exclusively within the family Solanaceae.

In testing a plant as to the susceptibility of its stem to this disease, usually a cutting was taken and placed with the stem immersed in three to four inches of water. The inoculum, in the form of mycelium bearing sporangia, or of a zoospore suspension, was then added to the water. After a few days at room temperature the stem of a susceptible host showed a brownish lesion extending from the water line upwards an inch or more. If the tissues of the stem collapsed in the discoloured region (Plate I, Fig. 13), the plant was considered to be susceptible. This method of testing plants for resistance or susceptibility does not closely simulate the conditions under which natural infections occur, and the environmental factors all favour the attack by the parasite on the host. However, plants that resist the disease under these conditions may be safely assumed to resist it under conditions less favourable for attack.

In some cases the reaction of leaves or fruits was tested by placing drops of zoospore suspension on the organ to be tested and keeping it in a moist chamber. Drops of sterile water were placed on similar organs that served as controls.

More than 40 commercial varieties of *Lycopersicon esculentum* Mill. were tested in this way. All showed complete susceptibility of stems with no appreciable difference between varieties.

The following species of *Lycopersicon* were also tested:

(1) *Lycopersicon esculentum* var. *cerasiforme* (Dun.) A. Gray (*Lycopersicum humboldtii* Dun.)
(2) *Lycopersicon hirsutum* Humb. & Bonpl.
(3) *Lycopersicon peruvianum* (L.) Mill.
(4) *Lycopersicon peruvianum* var. *dentatum* Dun. (*Lycopersicum chilense* Dun.)
(5) *Lycopersicon pimpinellifolium* (Jusl.) Mill.
(6) *Lycopersicum racemiflorum* Dun.*
(7) *Lycopersicum racemiforme* Lange*
(8) *Lycopersicum racemigerum* Lange*
(9) *Lycopersicon rhombifolium* Dipp.

The leaves of all species proved susceptible as did the stems of all except *L. hirsutum*, *L. peruvianum* var. *dentatum*, and *L. rhombifolium*. These three displayed a perceptible degree of resistance, though they were not immune. More extensive tests were made with *L. rhombifolium*. These showed that the same degree of resistance was inherited by the F_1 and part of the F_2 generations of a cross between it and *L. esculentum* var. Vetomold. The degree of resistance, however, was not sufficient to warrant continuing this phase of the problem for the purpose of breeding a tomato variety resistant to *Phytophthora parasitica*.

The tests were next extended to include as many plants belonging to the family Solanaceae as were available. Mature and young leaves and stems were used in most cases. In 16 of the 28 species tested, the leaves only were susceptible; in five only the young leaves were susceptible; while in the remaining seven all the leaves were resistant. In only five of the 19 species tested were the stems susceptible, the other 14 showing no reaction. In no case was a stem found to be susceptible when its leaves were resistant, but in many cases the leaves were attacked when the stem was not. The Irish potato is a striking example of the latter condition. Its leaves and petiole were as susceptible as tomato leaves, but all attempts to induce infection in stems by inoculating with zoospores or with mycelium in wounds failed.

The fruits of *Nicandra Physalodes* L., *Solanum Melonga* L., *S. nigrum* L., and *S. Pseudocapsicum* L. were tested and found to be susceptible to attack by germinating zoospores. Fruits of *Capsicum annuum* L. resisted this form of attack but were rotted by mycelium of the fungus placed in wounds.

While potato stems are immune to the disease, a tuber can be readily infected by placing drops of zoospore suspension on the surface or by inserting mycelium into wounds. The fungus develops quite rapidly, invading about two-thirds of the tuber within one week and causing the pink rot characteristic of certain *Phytophthora* species. The colour is not evident when infected tubers are freshly cut, but after exposure to the air for about 20 minutes a salmon pink colour develops over the area invaded. This area later becomes dark brown.

* *According to Muller (18) these species are synonymous with Lycopersicon pimpinellifolium.*

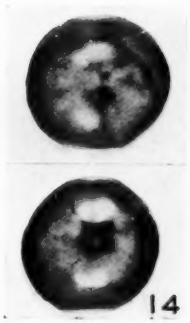
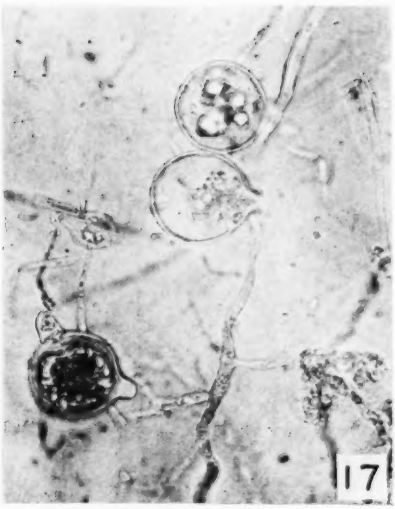


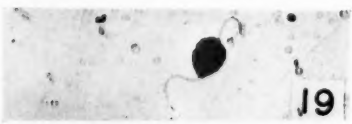
FIG. 10. *Typical lesions on stems of 6-weeks-old tomato plants naturally infected with Phytophthora parasitica.*

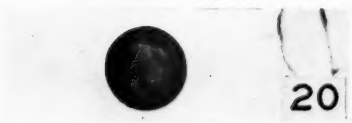
FIG. 11. *Extensive lesions on stems of mature plants: a, surface view; b, section showing breakdown of parenchymatous tissues.*

FIGS. 12 TO 14. *Tomato leaves, cuttings, and fruits, three days after inoculating with zoospores.*

FIG. 15. *Naturally infected fruit cut open to show depth of lesion.*







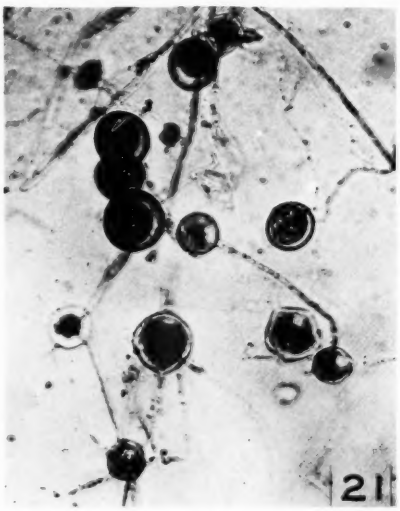


FIG. 16. *Sporangia of Phytophthora parasitica formed in association with a rootlet of a tomato seedling growing in water.*

FIG. 17. *Sporangia from culture on oatmeal agar. ca. 430×.*

FIGS. 18, 19. *Zoospores killed with osmic acid and stained with cotton blue. ca. 865×.*

FIG. 20. *Oospore formed on rootlet of tomato seedling growing in water. ca. 260×.*

FIG. 21. *Chlamydospores from culture on oatmeal agar. ca. 260×.*

A limited number of tests outside of the family Solanaceae revealed no susceptible host, with the single exception that the fungus caused decay in apple fruits following wound inoculations. Plants that showed no reaction included snapdragon, cucumber, dahlia, buckwheat, and garden bean.

The seedling reaction of a few plants to the parasite was tested by planting seeds in infested soil. Although the stem and leaves of petunia had proved to be immune, the seedlings of this plant damped-off readily. Seedlings of carrot, lettuce, radish, and pea were not affected. It was observed that young seedlings of chickweed (*Stellaria media* (L.) Cyrill) growing in a plot of infested soil in the field damped-off, apparently due to *P. parasitica*.

Several features of the infection capabilities of this isolate of *Phytophthora parasitica* are shown by these tests. It has a possible host range extending fairly generally throughout the family Solanaceae. It can attack storage organs such as fruits and tubers when other parts of the plant are immune. It parasitizes leaf tissue more readily than stem tissue. It can often infect young tissues when older tissues of the same type are resistant. This age resistance on the part of the host is shown in many cases by the difference in reaction between young and old leaves, and by the fact that only young succulent stems of tobacco can be infected.

Control

Since this disease is caused by a soil-borne parasite, the following measures would be expected to contribute to its control: (1) resistant varieties of host; (2) sanitation; (3) rotation of crops; (4) chemical seed treatments; and (5) soil disinfestation.

(1) *Resistant Varieties*

The most desirable form of control for this disease would be the development of a good commercial variety of tomato that would thrive in the presence of the pathogen, even under conditions favouring infection. However, the prospect of developing such a variety is not encouraging. While some resistance was found in certain wild *Lycopersicon spp.*, it was not a high degree of resistance even in mature plants and was apparently inoperative in their seedling stage.

(2) *Sanitation*

Unfortunately the presence of the pathogen is not detected until its effect on growing plants becomes apparent. While it is impossible at that time to rid the soil of the pathogen without injury to the crop, certain methods may be employed to reduce its destructive attacks. Remembering that it is soil-borne and that its spores are dependent upon water for their spread, one should avoid excessive watering and take every precaution to avoid splashing the soil onto the stems. Mulching the surface of the soil with straw will reduce the danger of this. So far as possible one should control the atmosphere of greenhouses so that it does not favour infection. In other words, greenhouses should be kept as cool and as dry as is feasible for satisfactory growth

of the plants. Dead plants should be carefully removed and destroyed. The tracking of soil from one house to another should be avoided as far as possible.

(3) *Rotation of Crops*

Whenever it is feasible, it is wise to alternate tomatoes with other greenhouse crops. Continuous culture of the same plant tends to increase the degree of infestation of the soil by parasitic organisms specific to that host. *P. parasitica* is not likely to be soon "starved out" by this practice, since it has been found to be still active in soil in which no susceptible host has been growing for at least a year. The degree of infestation may be temporarily reduced, however, to the point where the disease does not seriously interfere with production.

(4) *Seed Treatments*

Chemical seed treatments have been reported by various investigators to control damping-off of seedlings. Horsfall (11) recommended red copper oxide, zinc oxide, and copper sulphate for protecting seedlings from *Pythium*, *Rhizoctonia*, and *Phytophthora*. Semesan, a mercury compound, has also been recommended. Seed lots treated with these chemicals, as well as with copper chloride, copper carbonate, copper tartrate, copper naphthalate, copper acetate, and Copoloid (a colloidal copper compound) were planted in soil infested with *P. parasitica*. Some of the treatments, especially the Semesan, interfered seriously with the germination of the seeds. Most of the treatments reduced the amount of pre-emergence blight of the seedlings, but none of them gave adequate protection against postemergence killing. Since this is the more destructive phase of this disease, control by means of chemical seed treatment does not appear to be feasible. Moreover, since the parasite can attack plants at any stage, even to maturity, it is useless to try to protect the seedlings in the seed bed, then carry the fungus in the soil around their roots into the cold frame or greenhouse. How a little inoculum of this sort is sufficient to cause serious trouble was demonstrated by the experiments on the establishment and spread of the pathogen in the soil.

(5) *Soil Disinfestation*

The only completely effective means found for protecting tomato plants of all ages growing in seed beds, cold frames, or greenhouses is to disinfest the soil by some means of sterilization. The accepted practices of steam and formaldehyde treatments were found to be 100% effective. If it is available, steam is preferable, since planting can be done within a short time without danger of a chemical residue injuring the plants.

Two newer sterilizing agents not generally used were tried and found to be very effective. The first of these was calcium cyanamide, known commercially as Aero Cyanamid. To one flat of infested soil 20 gm. of pulverized Cyanamid were added and thoroughly mixed; to a second flat 40 gm. were added; a third flat was kept as a check. The above quantities are roughly equivalent to 1000 and 2000 lb. per acre respectively. No appreciable change

in the acidity of the soils was detected, all three lots showing a pH of 7.2 when tested three weeks after mixing. The flats were heavily watered for two weeks after the addition of the Cyanamid before they were planted with 500 seeds each.

Twenty-five days after planting the results were as follows:

Check flat:	340 germinated, 204 alive.
20 gm. Cyanamid:	409 germinated, 409 alive.
40 gm. Cyanamid:	391 germinated, 391 alive.

While both concentrations of Cyanamid controlled the disease, the higher concentration delayed germination greatly and caused considerable burning of the foliage, even to the extent of being lethal in many cases. The lower concentration delayed germination somewhat, but injury was confined to very slight burning at the tips of the leaves. The seedlings were a healthier colour and more vigorous than those in the check flat, apparently as a result of the increased nitrogen available. Perfect control without any injury could probably be obtained by waiting longer before planting or by using less Cyanamid.

The second chemical used to disinfest the soil was trichloronitromethane (CCL_3NO_2). This is known commercially as Larvacide, Chloropicrin, or "tear gas". One cubic foot of heavily infested soil was placed in a tightly closed wooden box. Two holes were made in the soil and 1½ cc. of liquid chloropicrin were poured into each. The holes were then filled and the surface of the soil was wetted to seal the gas in. The top of the box was sealed by pasting tarred paper over the cracks. After three days the lid was removed and the soil stirred to allow the gas to escape. Two weeks later one flat of this soil was planted with 500 tomato seeds, as was a check flat of infested soil.

Three weeks later the treated flat had 431 living seedlings and no dead ones. Only 120 of those in the check flat survived. Pre-emergence blight was also reduced, although the exact amount was not determined.

Both of these methods have possibilities as commercial treatments. Both are claimed by their manufacturers to kill nematodes and weed seeds as well. Cyanamid is in addition a fertilizer, but heavy doses unbalance the normal chemical composition of the soil and it is difficult to remove the excess which is injurious to plants.

Discussion

Several species of *Phytophthora* have been reported to be the cause of various diseases of tomatoes. *Phytophthora cryptogea* Pethyb. & Laff. was described by Pethybridge and Lafferty (20) in 1919 as the cause of a foot rot or collar rot. *P. infestans* (Mont.) de Bary, which causes late blight of potato, was shown by Reed (22) in 1912 to cause a similar blight in tomato. Mills (17) in 1940 demonstrated the relationship between the potato and tomato strains of this species. *P. mexicana* Hotson & Hartge was isolated from tomato fruits by Hotson and Hartge (12) in 1923. *P. terrestria* Sherb.

was described by Sherbakoff (25) in 1917 as the cause of "buckeye rot" of tomato fruits. Butler (4), Bewley (2), Ashby (1), Leonian and Geer (15), and Tucker (26) agree that this species is identical with *P. parasitica*. Zonate markings have not been observed in fruits infected with the organism used in the present study, and tomato stems inoculated with a *Phytophthora* species isolated from fruits showing typical buckeye symptoms did not become infected. Reddick (21) described a "stem-girdle" disease of tomatoes which affected seedlings, stems, foliage, fruit, and roots. The causal organism was identified by Tucker (26) as *P. parasitica* Dast. Reddick's description coincides very closely with that of the disease under investigation here. There are, however, differences in symptomatology, life history, and host range that suggest that the causal organisms are different strains of the same species. In the first place, root infections were much less severe and never resulted in the death of the plant in the present study. Secondly, Reddick reported that oospores developed sparingly on oatmeal agar. In three years of culture on various media, including oatmeal agar, no sexual stage has been found for any of the isolations made from tissues affected by the disease herein described, although on one occasion a few oospores were found in association with sterile roots of seedlings placed in infested water. Finally, Reddick reported successful inoculations of stems of bean, cucumber, and young egg-plants, whereas in the present study such inoculations failed to produce lesions.

The restricted occurrence of the disease raises an interesting question: Is the pathogen likewise limited in its distribution, or is it ubiquitous, but destructive only under a favourable combination of conditions? Referring to *P. parasitica* causing buckeye rot, stem girdle, and damping-off of tomatoes, Tucker (27) states that "distribution is probably general in the Gulf and South Atlantic states." A general distribution cannot be postulated for Ontario, although the pathogen can apparently survive in some soils here. Under normal conditions even where the organism is present in the soil this must be brought into the greenhouse before the fungus becomes aggressive. The studies reported here on temperature and moisture in relation to disease incidence account in part for this situation. In many of the local outbreaks an effort was made to trace the source of infestation. In every case it could be traced, directly or indirectly, to soil brought in from the field and used in the compost. In one case it was suspected that the organism was introduced with a load of muck soil, but it is not always a dominant inhabitant of such soil, since seedlings planted in muck from two different districts were not affected. In other cases the trouble began when grass sod was used in the compost. Sods of four different grass types were tested by growing tomato seedlings on them, but again no infection occurred. If the pathogen was present in these soils it must have been held at a very low level of aggressiveness by competitive and environmental factors under natural conditions. Greenhouse conditions are so unnatural, however, not only with respect to temperature and moisture but also to substrate, that the microbiological equilibrium must be changed drastically, and the new conditions may be

such as would favour the pathogen. Moreover, the common practice of raising two crops of tomatoes a year without rotation favours micro-organisms parasitic on this host.

The strain of *Phytophthora parasitica* causing this tomato disease is better able to compete with other micro-organisms in the soil than many of the soil-borne parasites that have been investigated in this respect. Henry (8) found that even a trace of non-sterile soil added to soil infested with *Helminthosporium sativum* P.K. and B. brought about a marked reduction in the severity of the foot rot disease in cereals, and that if larger amounts of non-sterile soil were added he was unable to recover the pathogen. The reverse situation is true of *P. parasitica*: a trace of infested soil is sufficient to cause a large amount of non-sterile soil to become infested. This parasite is not only more successful in establishing itself in the soil, but also in maintaining a level of activity that renders it destructive to its host over longer periods of time than many other parasites. For example, Sanford and Broadfoot (24) found that, in greenhouse culture, inoculum of *Ophiobolus graminis* that destroyed the first planting of wheat with take-all disease was wholly ineffective after 120 days on the second planting. The decline in degree of infestation of a soil by *P. parasitica* could be detected by repeated plantings of tomato seedlings, but the pathogen was still aggressive after one year in the soil. Furthermore, workers have frequently failed to get infection in the field when inoculum was applied to the soil, whereas *P. parasitica* has been shown to be virulent in field tests as well as in greenhouse experiments.

The effect of competition on the activity of *P. parasitica* is, nevertheless, appreciable, as shown by various experiments reported here. More information concerning the relationships of the other soil micro-organisms to this pathogen is highly desirable, though rather difficult to obtain. Henry (8) found that adding mixtures of bacterial cultures isolated from Edmonton black loam to soil infested with *Helminthosporium sativum* reduced foot rot disease in wheat slightly; soil actinomycetes suppressed the disease more than the bacteria; saprophytic soil fungi reduced the disease most of all and the pathogen could not be recovered from the soil 24 days after these were added to the soil. He attributed the reduction in the activity of *H. sativum* to exhaustion of food by the other soil fungi. Sanford and Broadfoot (24) studied the effects of fungi and bacteria isolated from the soil individually as to their ability to suppress *Ophiobolus graminis*. Of 26 species of fungi isolated, six gave complete protection of wheat seedlings, seven gave partial protection, while the remainder had no effect. Of 40 species of bacteria tested, 15 suppressed the parasite completely, eight gave partial protection, and the remainder had no effect. They also tested the filtrates from these cultures and found that some of them suppressed the virulence of the pathogen, though less effectively than living cultures. They believed that the toxicity of by-products of fungous and bacterial growth was the chief factor concerned. Broadfoot (3) cultured the same organisms with *O. graminis* Sacc. on potato dextrose agar and other media and found that some were antagonistic

to its growth while others were compatible. The former were not always the same ones that suppressed the activity of the pathogen in the soil, however. He thought that this difference in reaction might be accounted for by the fact that various micro-organisms, each with its complement of enzymes, produce substances that differ in kind and amount according to the substrate.

The effect of various plants residues on the aggressiveness of *P. parasitica* is in agreement with the observation of various workers that the microbiological equilibrium of a soil may be modified by changing the organic substrate. It is known that the addition of green manure to the soil brings about a striking increase in the bacterial population. The addition of green rye to certain soils has been found to reduce the amount of potato scab caused by *Actinomyces scabies* (Thax.) Güssow. Millard and Taylor (16) demonstrated that scab incidence was markedly reduced by the addition of grass clippings if the obligately saprophytic species *A. praecox* Millard & Burr was also present in the soil. The two species are incompatible in culture, the parasite making slower growth and apparently being starved by the saprophyte. Waksman and Hutchings (28) demonstrated associative and antagonistic relationships between micro-organisms concerned with the decomposition of plant residues. They showed that the type of decomposition is a function of the organisms of a mixed population that are in a position to attack the introduced substrate.

It would be of interest to know the effects of various oxygen and carbon dioxide tensions on the growth of *P. parasitica* as well as on the microfloral population as a whole. Garrett (6) postulated that high carbon dioxide concentrations, built up by the respiration of the micro-organisms and the roots of higher plants in the soil, constitute a primary factor in the suppression of *Ophiobolus graminis* by organisms better able to develop under those conditions.

The apparent dependence of *P. parasitica* on living roots of its host for migration through the soil is highly suggestive. Padwick (19) showed that *Ophiobolus graminis* spreads little, if at all, in bare soil, but it spread 12 inches in six weeks in soil in which susceptible grasses were growing. It apparently spread actively through the soil only by root contact, passing from plant to plant by means of the fine network of roots. Garrett (6) demonstrated, furthermore, that the spread of the fungus is along the outside of wheat roots, internal development being sharply restricted. *P. parasitica* was found in these studies to grow profusely around the roots of seedlings growing in water, and to be stimulated in its growth in liquid culture by the presence of rootlets. The soil in the immediate vicinity of plant roots or the rhizosphere is known to support a much higher microfloral population than that beyond the zone of the plant's influence. West and Lochhead (30) have shown that there is a qualitative as well as a quantitative difference here. They found that those bacteria that require the growth substances thiamin and biotin as well as amino nitrogen are favoured in the immediate vicinity of the roots. West (29) also demonstrated that the young roots of higher plants excrete significant amounts of thiamin and biotin normally, even under sterile conditions. Many

fungi, especially Phycomycetes, have been shown by Robbins (23) to respond to certain growth promoting vitamins, including thiamin. Leonian (14) found that substances, which he believed to be auxins, excreted by corn root tips, stimulated the growth of *Phytophthora cactorum* (L. and C.) Schroet. in culture. The production of some such substance or substances by the roots of tomato seedlings may well be the cause of the association observed between *Phytophthora parasitica* and the roots of its host.

Acknowledgments

The author wishes to express his sincere gratitude to Professor D. L. Bailey for suggesting and directing this investigation; to Professor J. W. MacArthur for providing many of the *Lycopersicon* and *Solanum* species used; and to Professor E. F. Palmer for placing at his disposal the facilities of the Horticultural Experiment Station, Vineland, Ontario.

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THE DETECTION OF CORAMINE[1]

BY G. H. W. LUCAS[2]

Abstract

Methods are described for the biological detection of coramine and for the production of coramine crystals with mercuric chloride. By the use of the crystal method as small an amount as 0.004 mg. of coramine has been detected.

This Laboratory has had to undertake the examination of horse saliva for the detection of various drugs used as stimulants and from the first has found pharmacological tests of great value. In the routine testing for strychnine, small frogs were injected with a purified extract of horse saliva. In some frogs it was noted that such an extract, which subsequently was proved not to contain strychnine, produced a peculiar type of convulsion and at times death. Suspicion was directed to coramine. Small young frogs were tested with small amounts of this drug and convulsions identical with those produced by the saliva from horses were observed.

Consequently, in the racing season of 1936 several experimental horses were given stimulant doses of this drug by mouth and a saliva sample was obtained. Later, when it was suspected that the drug was being given hypodermically to horses, the drug was administered in this way and again the saliva was examined after purification in the following routine manner.

Extraction of Coramine from Saliva

To the saliva and the washings of the swabs used in the collection of the sample, glacial acetic acid was added in an amount necessary to produce a concentration of 5% acetic acid. The mixture was then heated to 65° C. to precipitate some of the protein, and this was filtered out. The acid and the water were removed from the filtrate by heating *in vacuo* at 65° to 70° C. The dry residue was extracted five or six times with 10 to 12 cc. of boiling absolute alcohol. The combined extracts, centrifuged clear of protein, were warmed with a drop of 70% sulphuric acid, causing more protein to precipitate; this was removed by centrifuging. The supernatant liquid was evaporated to dryness on a steam bath under a fan. From the residue, taken up in about 1 cc. of water, the fatty material was removed by filtration through a micro filter made of asbestos. The filtrate, made ammoniacal,

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was extracted at once with 3 to 4 cc. of a mixture of 8% chloroform in ethyl acetate. Two or three extractions were made, and the combined extracts were evaporated to dryness on a steam bath. The residue was dissolved in ½ to 1 cc. of $N/20$ hydrochloric acid, the solution was made ammoniacal and re-extracted with pure chloroform. The chloroform extracts were evaporated on a steam bath and the residue was again taken up with a few drops of $N/20$ hydrochloric acid.

One portion of the final extract was examined by injecting it into a small frog after the hydrochloric acid was neutralized with sodium bicarbonate, while another was mixed with bichloride of mercury and set aside for crystalline tests. It was found that doses of coramine (given by mouth or subcutaneously), which produced stimulation in the horses, appeared in the saliva in amounts sufficient to cause convulsions in a small frog and to give crystals with bichloride of mercury. Hence the following experimental work was undertaken.

Pharmacological Test for Coramine

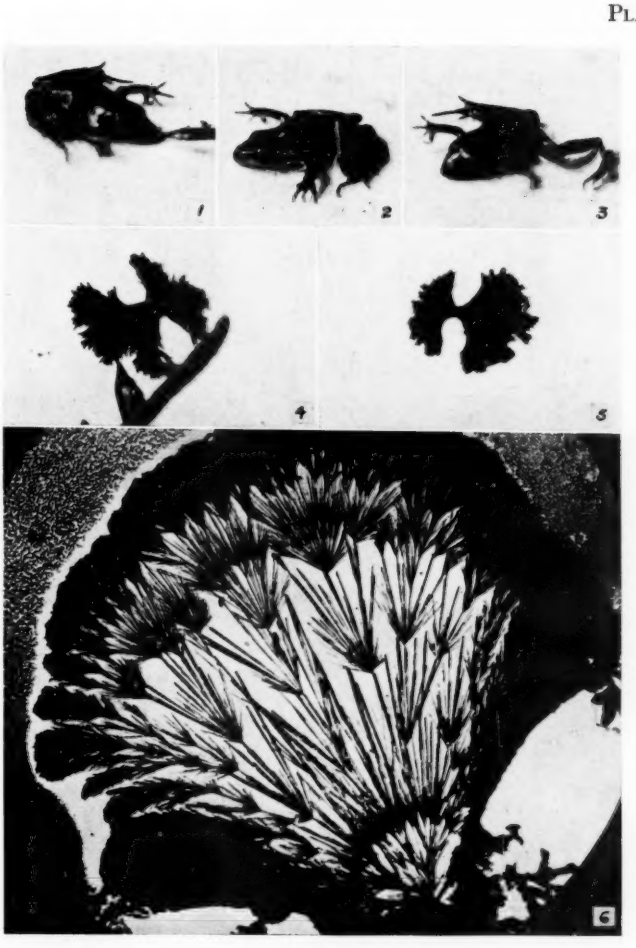
Using a fine hypodermic needle, varying amounts of coramine were injected through the floor of the mouth, into the abdominal lymph sac of frogs about 1 gm. in weight. About 1 mg. of coramine per gm. of frog produced the following marked reaction. A few minutes after injection the fore limbs began to stiffen. They were gradually extended to a right angle with the body, and the point of the chin fell to the supporting surface, a wetted porcelain plate. The pupils contracted and the eyeballs were withdrawn. The frog either died in about half an hour or eventually recovered. If a smaller dose (0.2 to 0.3 mg. per gm. of frog) was given, the frog after several minutes to a quarter of an hour appeared normal but, if stimulated by touching the point of its nose with a sharp point (needle) or by drawing such an instrument down its vertebral column, the front limbs were gradually extended stiffly and the above sequence of events occurred. Figs. 1, 2, and 3 show the reaction.

It was found that certain small frogs of about 1 gm. weight, when stimulated, reacted in the typical manner to 0.2 mg. of the drug. In most frogs weighing up to 30 gm., the response occurred with 0.4 to 0.5 mg. per gm. Without stimulation, all such frogs gave a typical marked response with 0.6 mg. per gm. or more. The reaction was produced in frogs 1 or 2 gm. in weight by a smaller amount of coramine per gm. than was required for large frogs.

Crystalline Test for Coramine

About the time that the pharmacological test for coramine was being developed, Mr. Charles Morgan, Chemist of the New York State Racing Commission, had discovered that coramine gave typical crystal forms with bichloride of mercury and he was good enough to write in regard to this. In this laboratory Mr. Morgan's observations have been confirmed.

Either with the extract from saliva prepared as indicated above, or coramine itself, typical crystals whose forms are shown in Figs. 4, 5, and 6 can be



EXPLANATION OF FIGURES

FIG. 1. *A frog in the early stages of coramine poisoning; the pen point touching the nose caused the convulsion to appear.*

FIG. 2. *The frog after convulsions are produced. This position is maintained for 10 min. or more, unless the frog is disturbed.*

FIG. 3. *The frog in the final stages of poisoning.*

FIGS. 4 AND 5. *Coramine crystals (sheaf-like) near bichloride of mercury crystals (×30).*

FIG. 6. *A sheaf of coramine crystals (×80).*

produced. If a drop of fluid containing 0.4 mg. of coramine or less is allowed to mix on a slide with a drop of a 5% solution of mercuric bichloride and evaporation is allowed to occur, the development of many typical crystals occurs. This first occurs towards the margin of the drop. Crystals of mercuric bichloride form and a little later, usually inside the ring of bichloride, the feathery crystals of the coramine mercuric compound develop. Their characteristic form is shown in the figures.

Acknowledgment

The author wishes to express his thanks to Prof. V. E. Henderson for his suggestions, advice, and criticism while this work was in progress.

THE DIAPAUSE AND RELATED PHENOMENA IN *GILPINIA POLYTOMA* (HARTIG)

III. BIOCLIMATIC RELATIONS[1,2]

By M. L. Prebble[3]

Abstract

The progress of intracocoon development in relation to temperature and moisture is described for a one-generation and a two-generation area in eastern Canada. In the one-generation area (central Gaspé) the degree of seasonal emergence from the overwintered cocoons can be forecast with considerable accuracy by means of sample analyses during the period of pronymphal and early pupal development. This is possible since few individuals that have not initiated development by late June do so later in the summer, even though environmental conditions are quite favourable. The technique fails in a two-generation area (south central New Brunswick) because members of the overwintered population may continue to respond to favourable temperature and moisture conditions throughout the entire season.

On the basis of biologic and climatic data the area occupied by the European spruce sawfly in eastern North America is divided into zones representing the probable distribution of one-, two-, and three-generation areas; intermediate transitional zones are also indicated.

The spruce sawfly encounters and has shown its adaptability to a considerable variety of climatic conditions throughout its distribution range in North America, producing a variable number of annual generations through variations in the diapause behaviour. The principal facts relating to seasonal development and climate, and a tentative division of the area occupied, or likely to be occupied in the future, into zones according to the number of seasonal generations, are outlined in this paper.

Climate and Development in a One-Generation Area

Climate

Most of the studies in a typical one-generation area were carried out in the valley of Berry Mountain Brook, one of the headwaters of the Cascapedia River in the Gaspé Peninsula, Que., at an altitude of 1500 feet above sea level. The region is characterized by high ridges, plateaus, and mountains to a height of 3000 feet or more and rather narrow valleys. Owing to the high altitudes and proximity to the sea, the climate is cool and the growing season short. Snow persists in the heavily wooded valley bottoms until late May or early June. Maximal daily temperature occasionally exceeds 75° F. in midsummer but the nights are cool. Freezing temperatures occur commonly up to mid-June and from early September onwards. Rainfall is

[1] *Manuscript received in original form March 29, and as revised, July 25, 1941.*

This paper is based on part of a thesis submitted to the Faculty of Graduate Studies and Research of McGill University, Montreal, Que., in April, 1940, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] *Parts I and II of this series appeared in the October issue.*

[3] *Assistant Agricultural Scientist, Forest Insect Investigations, Science Service, Dominion Department of Agriculture, Victoria, B.C.; on the staff of the same service, Fredericton, N.B., until 1940.*

frequent and abundant, and light snowfalls occur sometimes in September and commonly in October. Average temperature and precipitation data for June to October, based on records in the wooded valley bottom from 1932 to 1939, are summarized in the following synopsis.

	Mean air temperature	Mean soil temperature*	Precipitation, in.**
June	52.9	46.5	3.88
July	57.2	52.8	5.78
August	55.1	53.4	5.42
September	47.0	47.0	4.43
October (first half)	37.7	39.4	1.63

* *At depth of about 2 in. in moss.*
** *Includes rain and snow, the latter as 10 : 1.*

Development within the Cocoon

Development in the overwintered cocoons in central Gaspé begins soon after the snow disappears. Pronymphs are most numerous about mid-June, at approximately the same time that buds of white spruce and balsam fir burst. The first pupae appear about mid-June and the first adults late in the month. Emergence is usually heaviest in July though stragglers continue to appear until mid-August or later.

Two methods have been used in following the progress of development in overwintered cocoons. In the first, sample lots of cocoons are collected from the undisturbed moss at successive intervals, for immediate analysis. The method has the disadvantage that emerged sawflies cannot be included in the analysis, owing to the impossibility of distinguishing between empty cocoons of the current season and accumulated ones of former years. Consequently the highest percentage values of development (all stages beyond the eonymph) are obtained before adult emergence commences; the values drop as emergence progresses and approach zero when emergence is over for the season. Results of analyses in 1932 and 1935 appear in Table I.

The 1932 series indicated about 13% development in the stand under observation, an estimate corroborated by emergence of 220 adults (12.2%) from 1806 cocoons from the same stand kept for seasonal emergence. The estimated development from the 1935 series was about 26%, which was checked by 22.4% emergence from 2259 cocoons kept for seasonal emergence.

The second method consists of dividing a large collection of cocoons made late in the spring into sample lots of about 200, each of which is placed in a shallow wood and wire container under the moss. Successive lots are removed for analysis at intervals of two or three days, and, since adults and emerged cocoons are retained, the progress and percentage of development can be compared for all lots in the series. Data from extensive series studied in

TABLE I

ANALYSES OF PERIODIC COCOON COLLECTIONS, BLACK SPRUCE SLOPE, BERRY MOUNTAIN BROOK, GASPÉ

Time of collection and analysis	Number in sample	Pronymphs, %	Pupae, %	Unemerged adults, %	Total development*, %
1932					
June 21 – 27	376	6.7	6.9	0	13.6
July 2 – 5	444	1.8	5.6	1.4	8.8
July 7 – 13	306	2.0	3.6	1.6	7.2
July 18 – 23	356	1.4	2.8	3.1	7.3
July 26 – 29	395	0.8	0.5	0.2	1.5
Aug. 2 – 6	336	0.6	1.5	1.5	3.6
Aug. 8 – 12	251	1.6	0	0	1.6
Aug. 19 – 25	253	0	0.4	0	0.4
Sept. 1	151	0	0	0	0
1935					
June 21	139	7.2	15.8	3.6	26.6
June 28	198	0.5	16.7	5.6	22.8
July 4	206	2.4	5.3	7.3	15.0
July 10	156	1.9	2.6	9.0	13.5
July 19	153	1.3	0.6	0	1.9
July 26	122	0	0	0	0

* *Eonymphs, not shown in the tabulation, are complementary to the percentage of total development.*

1933, 1934, and 1939 are represented graphically, along with temperature and precipitation, in Figs. 1 to 4. In order to simplify the charting, no distinction has been made between living and dead, or emerged and unemerged adults; the final moult being considered the ultimate attainment in the course of development. Eonymphs, being complementary to the percentage of total development, have not been included in the graphs. As the figures are largely self-explanatory, little descriptive comment is necessary. In all but one of the series only eonymphs and pronymphs were present in the first samples analysed; pronymphs increased to maximum about June 15–20, then gave way gradually to pupae and ultimately to adults. Variations in the proportions of adults in the later samples and in the percentage of total development in all but the earliest samples, were entirely of a random nature. Hence the respective curves are indicated as straight lines of no slope at the mean for the series, starting when the characteristic mean value was first attained in individual samples. The curves for pronymphs and pupae decline gradually to the horizontal axis, disregarding the few individuals, most of which were dead, contained in late sample lots. The pertinent statistics for the four series are included in Table II. The slight discrepancy between the curves for adults and total development in each series was due in part to sampling errors (different numbers of sample lots figuring in the calculation

TABLE II

COMPARISON OF STATISTICS BASED ON SAMPLES OF COCOONS ANALYSED PERIODICALLY DURING THE SEASON, AND ON OTHERS HELD FOR SEASONAL EMERGENCE

	1933	1934	1934	1939
Forest type	Black spruce slope	Black spruce slope	White spruce flat	Black spruce flat
Figure showing analyses	1	2	3	4
Total number insects in series	7145	10,864	9913	9735
Number of samples	40	41	38	39
Total development, %	41.0	13.4	25.6	17.8
Basis, samples during period:	June 16 – Aug. 31	June 12 – Aug. 19	June 11 – Aug. 18	June 6 – Sept. 14
Number insects attaining adult stage, %	39.4	12.9	21.4	15.2
Basis, samples during period:	July 21 – Aug. 31	July 21 – Aug. 19	July 28 – Aug. 18	July 21 – Sept. 14
Check from lots held for seasonal emergence:				
Number of cocoons	1454	1759	2498	941
Emergence, %	35.0	11.0	22.8	16.3

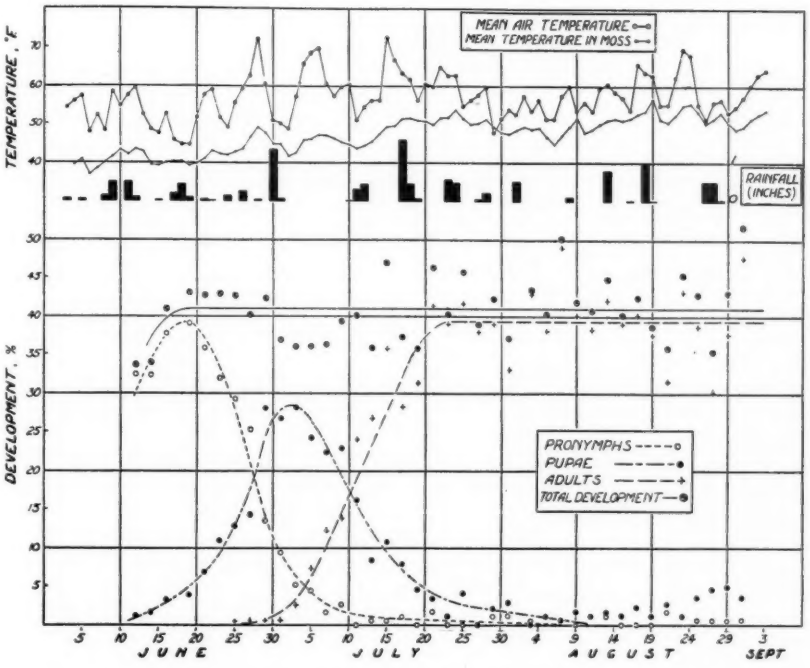


FIG. 1. *Progress of development within overwintered cocoons; black spruce slope, Berry Mountain Brook, central Gaspé, 1933.*

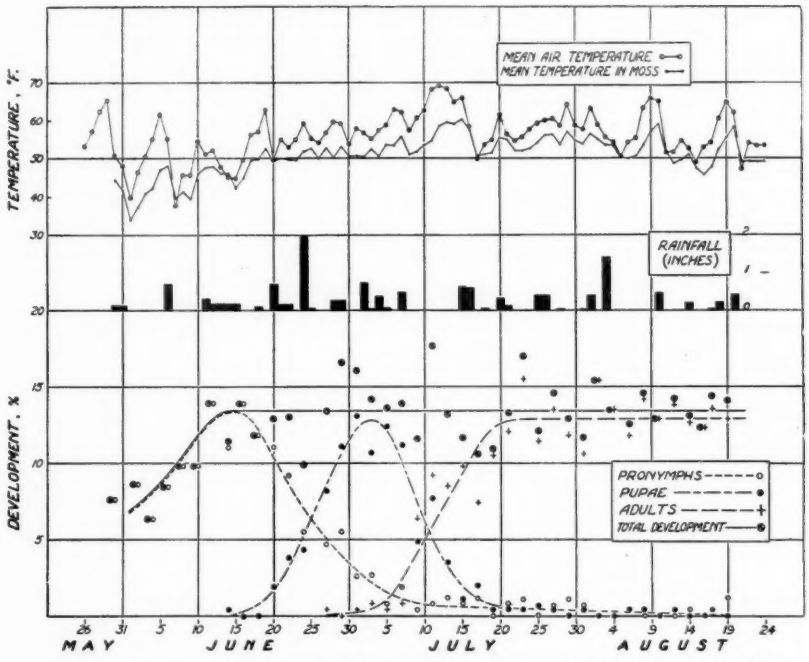


FIG. 2. *Progress of development within overwintered cocoons; black spruce slope, Berry Mountain Brook, central Gaspé, 1934.*

of the two curves), and in part to the death of a few pronymphs and pupae. The check values obtained from lots held for seasonal emergence (Table II) were very close to those resulting from the periodic analyses.

It is clear from the results of both methods of analysing development in overwintered cocoons, that a reliable estimate of the percentage of seasonal development in Gaspé populations can be obtained two to three weeks before emergence begins, and about five weeks before emergence is near completion for the season. This is so because very few individuals that have not resumed development by the latter half of June respond later in the season even though soil temperature is higher and precipitation abundant during July and August. One must conclude that the resumption of development in the Gaspé populations is controlled by factors operative before mid-June.

There is virtually no tendency for a second generation in central Gaspé even in the warmest seasons. Only two definite records of summer emergence from newly spun cocoons were obtained in eight years' studies, though over 18,000 reared cocoons and many times that number of collected newly spun cocoons were under observation. However, there is occasionally some development of newly spun eonymphs into pronymphs in the late summer, but

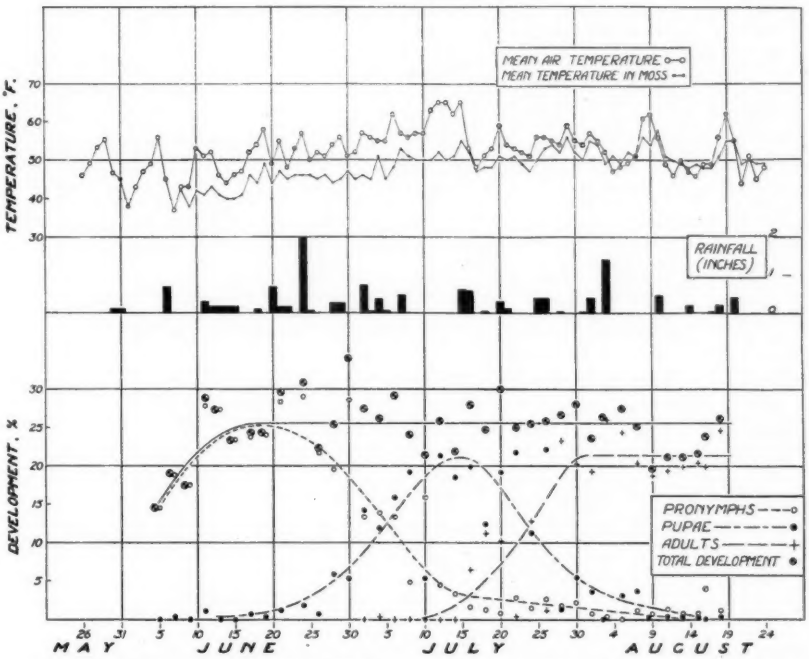


FIG. 3. *Progress of development within overwintered cocoons; while spruce flat, Berry Mountain Brook, central Gaspé, 1934.*

the pronymphs* either enter into diapause that persists for several months at optimal conditions, or are inactivated by cold, and in either case their further development is impossible until the following spring. In this respect the spruce sawfly differs from *Lyda hypotrophica* Hartig (9) and *Lygaeonematus pini* Retz. (7), which undergo considerable development within the cocoon during the winter.

Climate and Development in a Two-Generation Area

Climate

Studies in a typical two-generation area have been concentrated in the Fredericton district of south central New Brunswick. The region is moderately rolling with broad valleys and low hills, few of which exceed 500 feet above sea level. Snow disappears from the spruce woodlands in late March or April, though occasionally it lasts until early May. Freezing temperatures are infrequent after the last of April, or before early October, and maximal temperatures of 80° F. or higher are common between late May and early September. Normal climatic data for Fredericton are included in the following synopsis.

* *These possibly represent emergent strains which, as noted elsewhere, cannot be wholly eliminated in a strictly one-generation area.*

	Mean air temperature	Mean soil temperature*	Rain	Snow	Total precipitation
January	13.1	—	1.51	23.6	3.87
February	14.6	—	0.91	23.0	3.21
March	26.0	—	2.01	16.3	3.64
April	38.7	36.2**	2.19	7.0	2.89
May	50.9	42.9	3.11	0.2	3.13
June	60.2	53.3	3.70	0	3.70
July	66.0	58.7	3.56	0	3.56
August	64.1	59.4	3.98	0	3.98
September	56.0	52.7	3.50	0	3.50
October	45.5	43.0	3.98	0.4	4.02
November	32.5	—	3.04	8.0	3.84
December	18.9	—	1.53	19.0	3.43

* *Based on five seasons' records taken under the moss in spruce–balsam woodlands.*
** *Record for April of one year only.*

The growing season is close to two months longer, and the mid-seasonal mean air temperature about nine degrees higher, than in central Gaspé.

Development within the Cocoon

Development within the overwintered cocoons in the Fredericton district starts in late April or early May. The first pupae appear about mid-May and the first adults late in the month. Emergence is normally ended by July,

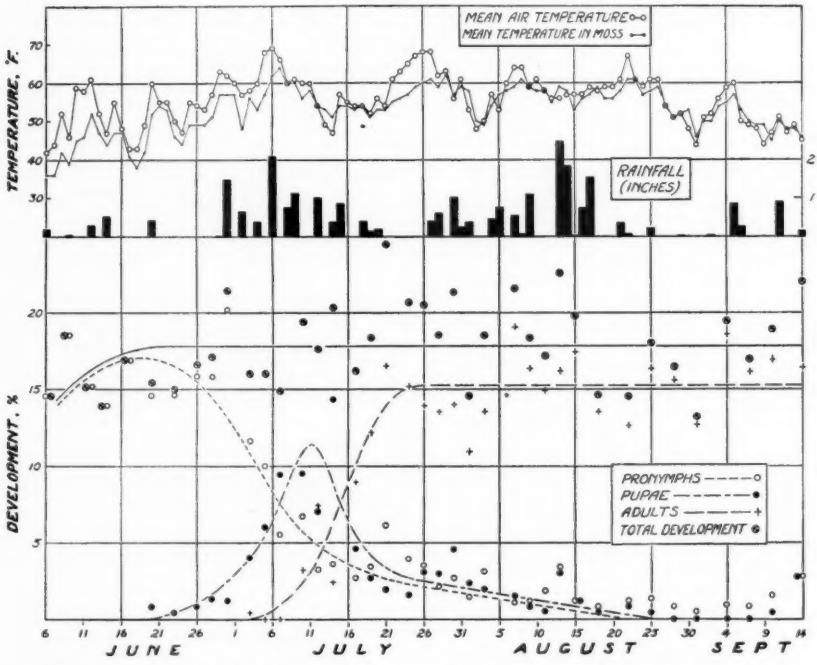


FIG. 4. *Progress of development within overwintered cocoons; black spruce flat, Berry Mountain Brook, central Gaspé, 1939.*

but in the event of unusually dry spring weather the resumption of development in many of the overwintered cocoons is retarded. Due to the capacity of the overwintered eonymphs to respond to moisture later in the season it is not possible to forecast the percentage of development by the methods so satisfactory for a one-generation area. This is clearly shown by the analyses (Table III) of samples of overwintered cocoons in 1939, a season with dry weather in late May–June, and in late July–August, and with abundant rainfall in September and October. It is significant that many eonymphs resumed development in the latter two months.

TABLE III

PERIODIC ANALYSES OF SAMPLE LOTS OF OVERWINTERED COCOONS, ENGLISH SETTLEMENT, YORK CO., N.B., 1939

Date of analysis	Number in sample	Pronymphs, %	Pupae, %	Unemerged adults, %	Emerged adults, %	Total development*, %
May 14	150	18.7	0	0	0	18.7
May 27	218	19.7	2.8	0	0	22.5
June 4	299	12.1	6.0	0	0	18.1
June 10	285	11.9	12.3	0.7	0	24.9
June 17	295	12.2	10.5	2.4	0	25.1
June 24	304	8.9	11.8	8.6	1.0	30.3
July 1	278	4.7	7.9	1.4	6.5	20.5
July 8	247	6.5	6.1	3.6	7.7	23.9
July 21	312	7.0	7.0	2.6	19.9	36.5
Aug. 6	278	14.4	14.0	8.6	24.5	61.5
Aug. 12	265	9.1	11.3	6.0	26.0	52.4
Aug. 19	307	8.5	3.6	1.6	29.0	42.7
Sept. 1	298	10.4	10.7	4.4	39.3	64.8
Oct. 10	314	30.9	7.3	3.5	35.0	76.7

* *Eonymphs, not shown in the tabulation, are complementary to the percentage of total development.*

Although a considerable loss in productivity results from dry spring weather, the latter does not prevent the occurrence of a fairly strong second generation, other conditions being favourable.

Zones with Different Numbers of Generations*

In addition to central Gaspé and south central New Brunswick, knowledge of seasonal development is available also for the Parke Forest Reserve, Kamouraska Co., Que.; for the Timiskaming district of Quebec and Ontario, where a partial second generation is produced (1); for southwestern Maine, where a partial third generation may occur (8); and for Connecticut where three generations are produced (4, 5). Information from points distributed throughout the range of the insect in eastern Canada has been obtained from the Forest Insect Survey. These combined records in conjunction

* *Dr. A. W. A. Brown, Entomologist-in-Charge, Forest Insect Survey, Division of Entomology, Ottawa, is joint author of this section.*

with meteorological data for many stations, permit the division of the occupied and adjacent areas in eastern North America into zones in which a different number of seasonal generations may be expected.

Meteorological data were accumulated for 137 stations in Canada and for 104 stations in the United States, distributed in the area bounded by the 51st parallel of latitude, the 82nd meridian of longitude and the Atlantic Ocean, including the whole of the present known range of distribution of the insect in North America. In view of the relative unimportance of rainfall as a factor influencing the number of generations in areas where most intensive studies have been conducted, the climatic analysis has been restricted to temperature characteristics alone.

The first question to be settled is that of a temperature index satisfactory for the description of each locality. With so many localities necessary for adequate representation of the large areas involved, it has been impossible to deal with daily temperature data; consequently monthly summaries have had to be used although the writers are well aware of their limitations. The temperature index must also be restricted to the developmental season, since winter conditions have no relation to sawfly development. Seasonal mean temperature is unsuitable as an index since it fails to take account of the length of the season. The only reasonable approach is to work out an index including both temperature and time, in the manner so long ago used by Merriam (6) in the enunciation of the laws governing northward distribution of plants and animals. Shelford (10) attacks Merriam's method, which was the accumulation of daily mean temperature above a theoretical threshold, on several grounds: (1), the erroneous summing above 32° F., instead of 43°, which was the theoretical threshold, thus invalidating the conclusions but not necessarily the method; (2), the failure of day-degrees calculated from a daily mean to coincide with or in many cases even to approach values based on hourly or two-hourly means; and (3), the failure to recognize that the effect of one degree of temperature is not necessarily the same at all points of the temperature scale. Moreover, Kendeigh (3) has shown the method to be basically erroneous in relation to birds and mammals, which through regulation of body temperature and modification of the micro-climate (nest temperature in the case of birds) are to a large extent independent of environmental temperature above a threshold or toleration point.

With these considerations in mind, proposals involving the use of accumulations of monthly means above a given base are made with full realization of the lack of scientific precision, though the procedure is not without precedent. (Hopkins (2) uses the method for arriving at the sum of effective temperature for record localities in bioclimatic studies.) The justification for the use of the method in the present instance is that deductions based on analysis of climatic data are in accord with the facts in a number of localities for which biological data are available.

The same threshold, viz., 43° F., as that employed by Hopkins has been used here. This is very close to the threshold value for development within the

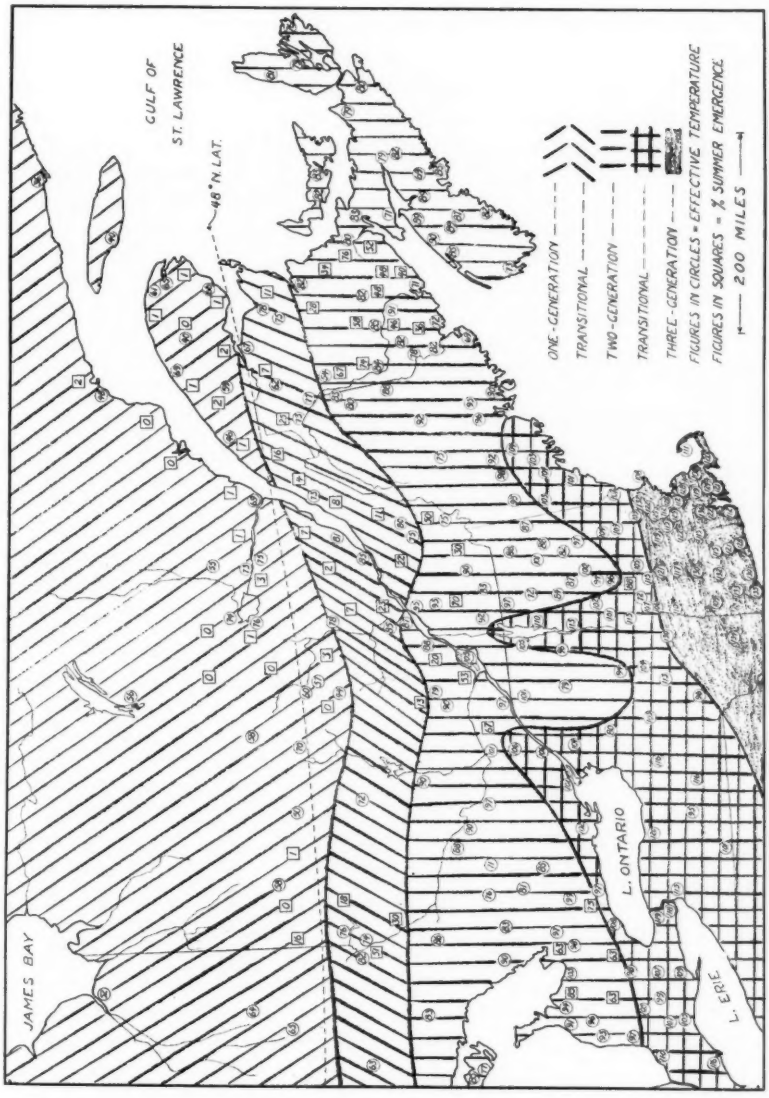


FIG. 5. *Map of northeastern North America showing division into zones according to number of seasonal generations. (Method of zonation described in text.)*

cocoon, for incubation, and for larval feeding though admittedly a monthly mean of 43° is an abstract value. Monthly mean temperature above this base was accumulated for each locality for which climatic data were obtained, and the sums are shown on the accompanying map (Fig. 5).

The use of the sums of "effective monthly temperature" in zoning areas characterized by variable numbers of seasonal generations of the spruce sawfly rests on the following argument.

1. The sum for central Gaspé based on monthly normals is 40; based on a very favourable year (1937) it is 49; and based on a very unfavourable year (1936), but one with survival of a small proportion of the larval population, the value is 33.

Other areas known to have but a single generation, or at most an entirely insignificant emergence for a second generation not normally surviving, have somewhat higher sums: Parke Reserve, 60; Causapscal, 59; Gaspé Harbour, 65.

2. The sum for the Fredericton district based on monthly normals is 85; based on a very favourable year (1937), 101; and based on a very unfavourable year (1936, small second generation), 79.

The sum for various localities in the Timiskaming district and in northern New Brunswick, known to have a small second generation, averages about 72 to 73.

3. The sum for the three-generation area in Connecticut, based on nine localities in the State, averages about 124.

Extensive biological data are also available to aid in the zoning of areas characterized by variable numbers of seasonal generations. Although intensive field studies have been carried out in only a few localities, records on developmental behaviour have been obtained from survey samples collected throughout the entire range of distribution in Canada. The studies in south central New Brunswick have shown that the earlier cocoons of the first generation yield a high summer emergence, later ones practically none, even though temperature conditions are not sensibly different at the time of spinning. This suggested the possibility of characterizing Canadian localities from which sample lots originated, as one-generation, two-generation, or transitional areas (the latter with a small second generation, relatively unimportant), by analysis of emergence in survey samples received by the Forest Insect Survey. The analysis has been restricted to those samples containing cocoons spun by early August, except in the case of samples originating in northern areas (the 48th parallel of latitude has been taken as an appropriate demarcation) where development is so much later that very few or no cocoons are spun by that time. Consequently the analysis of samples from northern areas has been extended to all samples of the season. The percentage values of summer emergence shown on the map (Fig. 5) are based on the pooled results of four years' survey records at Ottawa, one year's records at Fredericton, and one year's records at Duchesnay, Que. Owing to the light infestations throughout

much of the distribution range, and the consequent small samples, results for contiguous localities (within a radius of about 35 to 40 miles) have been combined. The dependability of the calculated values, as a measure of the developmental behaviour of the populations from which the samples arose, can be evaluated in a number of instances.

1. Emergence values of 1 to 2% were obtained for samples from the coastal areas of the Gaspé Peninsula. Although a second generation does not occur naturally, it is not unexpected that a small emergence would result in material reared during the latter part of the larval development at the more favourable climatic conditions at the Forest Insect Survey headquarters. In fact, the results almost exactly duplicate those reported in a previous section, where continuous rearing of central Gaspé stock, from the egg stage onwards, at Fredericton gave a 1% emergence value.

An emergence value of 4% was obtained from samples originating in Kamouraska County, Que., known to be an area in which one generation is the rule, with occasionally a very small partial second generation.

2. Although results for individual localities in the Timiskaming district were quite variable, due partly to small samples, the combined data for all localities and years indicate an emergence value of about 33%, corresponding closely to Dr. Atwood's field determination of 30%.

3. Emergence values of 7 to 25% were obtained for samples from northern New Brunswick, known to have only a partial second generation; and of 28 to 76% for samples from central and southern New Brunswick, where a strong second generation is the rule.

The various zones were defined as follows:

One-generation zone: sum of effective temperature not exceeding 65; emergence value not exceeding 3%.

Transitional zone: partial second generation normal or occurring in exceptionally favourable seasons: sum of effective temperature, 66 to 80; emergence value usually under 25%.

Two-generation zone: sum of effective temperature, 81 to 100; emergence value usually over 40%.

Transitional zone: partial third generation: this zone is established only tentatively, based on the probability that a partial third generation occurs in areas climatically similar to southwestern Maine; sum of effective temperature, 101 to 115.

Three-generation zone: sum of effective temperature, in excess of 115.

The zone boundaries shown on the map (Fig. 5) were established by interpolation, an attempt being made to strike a balance between climatic and biologic data where these were not in accord. A few examples will suffice to explain how this was done. A fairly high sum of effective temperature, i.e., 88, indicative of a two-generation area, is characteristic of the Berthier district of Quebec, while the emergence value based on survey samples (20%)

is not sufficiently high to warrant the classification of the district as a two-generation area. But since the samples were not extensive, greater weight was given to the climatic data. A few examples where the climatic data were not used in the final zonation include, *(a)*, Sidney, Saint John, and Eastport, coastal towns with effective temperature sums of 76, 71, and 69, respectively, which are dissimilar to localities only a few miles inland; *(b)*, large cities such as Quebec and Montreal, with effective temperature sums of 85 and 105, because summer conditions in large cities are known to be non-representative of the surrounding region; and *(c)*, localities of exceptional temperature sums compared with neighbouring points, due to altitude or other local features, e.g., Plaster Rock, Victoria County, N.B., with an unusually low sum of effective temperature, i.e., 54, for an area known to be a two-generation area. Other discrepancies may also be noted, since it is obviously impossible to attempt a classification of the large area except on the basis of regionally characteristic indices.

It is of especial interest to point out that the Saguenay valley of Quebec was originally classified as a transitional zone in which a partial second generation may be expected. This zonation was supported by the (normal) climatic indices, and by the biologic data prior to 1940. The biologic data based on more extensive samples in the unfavourable season of 1940 were not indicative of a partial second generation, and were of sufficient weight to depress the average emergence value to 3% or less. Therefore this area is not indicated as a transitional zone in the present classification, though it is possible that in reality the area is transitional in the strict sense, having a partial second generation only in the most favourable seasons.

The writers anticipate that corrections in the zonation will probably be necessary as knowledge of the regional behaviour of the spruce sawfly increases. In the meantime the proposed zonation will serve to focus attention upon areas doubtfully classified at present, and upon various aspects of the bioclimatology requiring further study.

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LA DIAPAUSE CHEZ LES TENTHRÈDES

PARTIE I[1]

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Sommaire

Le présent travail comprend une étude sur l'action de certains facteurs sur la rupture de la diapause chez les Tenthrèdes entre autres *Diprion polytomum* Htg. et *Pristiphora erichsoni* Htg. La température optimum d'hibernation serait aux environs de 32° F. Aucune des espèces étudiées n'a pu vivre plus de 10 semaines à 0° F. Le milieu optimum de nymphose pour *Diprion polytomum* serait de 75° F. à 80° F. avec une humidité relative de 85% et plus. Les chocs thermiques au début de l'hibernation provoquent des diapauses plus difficiles à rompre alors que les mêmes chocs à la fin de l'hibernation activent la réactivation. A des températures voisines du point de congélation, les larves peuvent vivre plus de trois semaines complètement submergées dans l'eau. De courtes immersions dans l'eau durant l'hibernation augmentent le pourcentage d'émergence. Le métabolisme est encore plus intense si les immersions se font dans une solution d'acide sulfurique à pH 3. Par contre le glycocolle inhibe la réactivation et augmente le taux de mortalité.

Introduction

La diapause chez les insectes présente un intérêt scientifique indéniable, aussi a-t-elle suscité de très nombreux travaux. Par ailleurs, ce problème est fort important au point de vue économique et, en ces dernières années, il est même devenu d'une grande actualité dans l'Est du Canada, à la suite de la découverte en notre pays de la mouche à scie européenne de l'épinette, *Diprion polytomum* Htg. Cet insecte, comme chacun sait, a envahi presque toute notre province et, à date, il a causé des dégâts considérables à nos forêts d'épinette. Or ce qui est le plus intéressant dans le comportement de cette mouche à scie, c'est le fait qu'elle peut avoir deux et même trois générations dans certaines régions, tandis que dans les forêts du Nord, elle n'en a qu'une et, de plus, la pronymphe peut rester en diapause dans son cocon six et même sept ans avant de donner naissance à un adulte.

Evidemment, cette longue diapause présente des inconvénients mais aussi des avantages pour la survivance de l'espèce. Il est vrai que des individus subissant un arrêt de plusieurs années dans leur développement auront un potentiel biotique beaucoup plus bas que s'ils avaient deux générations ou plus par année. Il est non moins vrai que dans la partie nord de son habitat, les principaux ennemis de *D. polytomum*, les musareignes et les écureuils, seront des agents de contrôle plus efficaces, la pronymphe étant à leur merci durant une plus longue période. D'un autre côté, certaines méthodes de lutte chimique ou sylvicole, de même que certains facteurs naturels de réduction

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auront moins de succès parce que n'affectant qu'une partie de la population, l'autre étant au repos dans le sol. Ainsi durant l'été 1940 une maladie infectieuse a réduit la population larvaire de façon substantielle dans la Gaspésie (49). Mais son effet sur la population future de l'insecte sera loin d'être aussi sensible qu'il le fût, par exemple, dans le Vermont et le New Hampshire, alors qu'à certains endroits la mouche à scie fût à peu près éliminée, apparemment par la même maladie (33). Pour toutes ces raisons, une étude des facteurs pouvant affecter la diapause de la mouche à scie européenne de l'épinette est donc très importante.

Pour cela, j'ai d'abord cherché à établir l'influence de différentes températures d'hibernation et de nymphose afin d'en arriver à la température optimum pour chacun des deux états. A ces températures optima et pour différentes périodes d'hibernation, j'ai fait agir, soit au cours de l'hibernation, soit durant la nymphose, soit encore durant ces deux périodes, d'autres facteurs ou agents susceptibles de provoquer la rupture. Puis, à l'aide d'analyses statistiques, j'ai pu évaluer et comparer l'influence de chacun de ces facteurs ou agents dont les principaux furent les suivants: variations brusques de température ou chocs thermiques; atmosphère desséchée et très humide; immersions dans l'eau; immersions dans des solutions acides à des pH comparables à ceux qui existent dans le sol*; immersions dans de l'acide sulfurique concentré*.

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Les expériences préliminaires à ce travail furent exécutées au Collège Macdonald au cours de l'hiver 1938–1939 sous la direction du Prof. Melville Du Porte, dont les conseils me furent alors très utiles. Il me fait plaisir de lui en exprimer ici toute ma gratitude.

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Historique

Le terme diapause fut employé pour la première fois par Wheeler pour désigner une période de repos durant le développement embryonnaire. Plus tard, Henneguy a étendu le sens de ce mot à tout arrêt dans le développement ontogénétique d'un animal, depuis la fécondation de l'œuf jusqu'à l'âge adulte. Nous aurions donc ainsi des diapauses embryonnaires, larvaires, et nymphales.

Les mots hibernation et estivation sont aussi employés pour signifier les diapauses survenant durant l'hiver et l'été. Shelford (106) et Bodine (18), cependant, font une distinction entre la diapause et l'hibernation. Il faudrait

* *Ceux-ci seront examinés dans la Partie II de cet article.*

appeler hibernation les cas où l'arrêt dans le développement est causé par la température seulement (Bodine) ou par des conditions défavorables (Shelford). La diapause, d'un autre côté serait inhérente à l'espèce (Bodine) et le développement arrêterait spontanément (Shelford). Ce serait là une distinction très importante s'il était prouvé que la diapause est un phénomène héréditaire ou spontané, mais plusieurs auteurs ne partagent pas cette manière de voir.

La diapause est un sujet qui a été très discuté et sur lequel des opinions variées ont été émises. Athanasiu (2) en 1909 fit une revue très complète des recherches exécutées jusqu'au début de notre siècle sur ce qu'on appelait alors l'hibernation ou l'estivation. Plus récemment, Cousin (30) dans son étude sur le même sujet a résumé de façon substantielle les travaux publiés sur la diapause antérieurement à 1930. C'est pourquoi, je ne donnerai ici qu'un aperçu des plus importantes et des plus récentes théories. Pour plus ample information, on consultera avec avantage les auteurs ci-dessus mentionnés.

Les principaux facteurs et organismes qui ont été mis en cause pour expliquer la diapause chez les insectes peuvent être classés comme suit: *a.* les glandes hormonogènes; *b.* l'hérédité; *c.* les facteurs internes; *d.* les facteurs physiques ou externes.

Wigglesworth (120–121) a beaucoup étudié l'influence des hormones sur le développement des insectes. Après avoir opéré des nymphes de *Rhodnius prolixus* et leur avoir enlevé l'hormone provoquant les mues, le "corpus allatum" situé dans la tête, il a montré que les sujets ainsi traités ne croissaient pas du tout, même après avoir absorbé un repas complet. De tels individus sont en diapause; et la croissance ou la métamorphose n'aura lieu que si l'hormone requise est introduite dans le sang. Ceci dit-il suggère naturellement que la diapause chez les autres insectes, *quand elle n'est pas occasionnée par les effets directs du milieu*, peut résulter d'un affaiblissement temporaire de l'hormone promouvant la croissance.

Parmi les auteurs considérant la diapause comme héréditaire ou rhythmique, mentionnons simplement: Babcock (3, 4), Baumberger (10, 11), Bodine (15–21), Holmquist (53, 54), Pictet (77–82), et Readio (86). Tous admettent, cependant que cette qualité inhérente peut être modifiée à un plus ou moins grand degré par les facteurs du milieu.

Parker et Thompson (68) ont associé la diapause de la pyrale du maïs avec le développement des organes reproducteurs. Au printemps, certains changements définis se produisent chez les rudiments d'organes génitaux. Ils ont constaté quatre phases de développement des gonades mâles, et la deuxième phase serait le début d'une activité physiologique qui, suivant le cas, élimine ou met fin à la diapause. Cependant, comme l'a fait remarquer Cousin, cet état particulier des organes génitaux peut caractériser la diapause de *Pyrausta nubilalis*, mais n'en est pas le facteur déterminant et les causes doivent être cherchées ailleurs. Les mêmes remarques s'appliquent au processus enzymatique étudié par Townsend (114) durant l'hibernation de la pyrale du pommier *Carpocapsa pomonella*, et le peu de catalase noté par Spooner (109)

chez le même insecte. Tous ces changements, tout comme la réduction dans les échanges respiratoires (74–75), la perte d'eau (101), et de gaz carbonique (116), sont des modifications prenant place durant l'hibernation et caractérisant la diapause, mais, apparemment, n'en sont pas les causes déterminantes.

Nombreux sont ceux qui ont attribué la diapause à une intoxication de l'organisme. Suivant les uns, tels, Baumberger (11), Bellion (13), Child (27), Dubois (35–37), Kamenskii et Paikin (57), cette intoxication serait le résultat d'accumulation de gaz carbonique dans l'organisme, alors que pour d'autres (91–97), elle serait liée à une condition de surcharge de produit d'excrétion. Plus récemment, Roubaud (98) a attribué l'hibernation obligatoire de la mouche *Phlebotomus pappatasii* à l'influence exercée par l'organisme maternel. Lorsque les ovules sont retenus dans les ovaires pendant un temps anormal, ils s'y surchargent d'éléments inhibiteurs du développement. Les effets de ce blocage se feront sentir ultérieurement amenant la diapause. D'après lui il s'agit bien là "d'un effet de surcharge métabolique, puisque le forçage à la chaleur entraîne habituellement la mort. C'est la détente prolongée, assurée par le repos de l'hibernation, qui permet aux individus asthéniques de ranimer leur activité." L'hypothèse de Bodine (15–21) se rapproche beaucoup de celle de Roubaud. La plupart de ses expériences ont eu pour sujets des sauterelles. Pour expliquer la diapause des œufs de *Melanoplus*, il introduit l'expression "diapause factors" ou facteurs de diapause, qui peuvent être soit chimiques, physico-chimiques ou encore des facteurs physiologiques–génétiques, présents dans l'œuf en quantité plus ou moins constante lors de l'oviposition. Suivant Bodine, ces facteurs "x" sont extrêmement sensitifs aux basses températures, au-dessous du zéro ou seuil de développement, et peuvent être complètement détruits ou supprimés.

Il n'y a probablement pas de facteur qui ait été plus étudié que la température. C'est pourquoi, pour éviter toute longueur, je me contenterai de mentionner ici les travaux d'Abeloos (1), Ball (9), Balachowsky (6), Brumpt (23), Sanderson (103), et Volkonsky (118). Quant à l'humidité, la majorité des auteurs reconnaissent que ce facteur joue un rôle important avec la reprise des activités après l'hibernation. D'autres expérimentateurs vont plus loin et croient que pour certains insectes l'humidité est le facteur de premier ordre sinon le seul concerné avec la diapause.

Des résultats très intéressants furent obtenus par Sabrosky et ses collaborateurs (102) en soumettant les œufs de la sauterelle, *Acridium arenosum angustum* à l'influence de radiations électriques blanches et violettes. Normallement cette sauterelle hiverne à l'état d'œuf, mais au moyen de ces irradiations, ils ont pu obtenir une génération d'hiver et briser la diapause, alors que la chose était impossible en soumettant simplement les œufs aux températures élevées de la serre. Par ailleurs, Hufnagel et Nabias (55) ont montré que l'action du radium n'apporte aucune modification dans le développement des Calligrapha et Hyponomenta, et Ray (85) a constaté que des irradiations de ½ à 4 heures retardaient le développement de *Melanoplus differentialis*

et pouvaient même avoir un effet mortel. Ceci concorde avec les expériences de Hussey, Thompson, et Calhoun (56) qui ont trouvé que chez les larves de *Drosophila*, la durée moyenne de la période prénymphale est une fonction croissante de la durée d'irradiation aux rayons X.

Dans ce qui est probablement le travail le plus approfondi et le plus complet sur ce sujet, Cousin (30) soutient que la diapause est le résultat d'une perturbation physiologique spécifique en liaison directe ou indirecte avec le séjour de l'insecte dans des milieux défavorables.

Ses expériences avec *Lucilia sericata*, démontrent que les conditions brisant la diapause sont généralement liées très étroitement avec celles qui l'ont déterminée. Ainsi une diapause causée par une sécheresse peut être rompue par un séjour dans un milieu où l'air est saturé de vapeur d'eau. Mais lorsque la durée de la diapause a été assez longue ou que les facteurs qui l'ont provoquée ont agi de façon très intense, cette relation entre la cause de la période de repos et la reprise du développement n'est pas aussi évidente. Pour obtenir la rupture de la diapause, il peut être alors nécessaire d'adjoindre au facteur qui l'a occasionnée, d'autres provoquant certains chocs et jouant le rôle d'excitant.

Matériel et Méthodes

Provenance des individus

Pour mes études préliminaires, saison 1938–1939, je me suis servi de cocons provenant des échantillons reçus au cours de l'été en rapport avec notre inventaire relatif aux insectes forestiers (48). Nous avions des spécimens nous venant d'à peu près tous les comtés de la Province et pour chaque individu une fiche donnant entre autres détails: son identité, sa plante nourricière, et la date de formation du cocon. J'espérais ainsi pouvoir montrer l'action du climat, de l'alimentation, et des conditions saisonnières sur la durée de la diapause, mais ce me fut impossible; les renseignements que je possédais étant de nature trop générale. De plus, mes sujets venant de différentes localités et ayant passé une partie de leur vie dans leur milieu naturel, certains facteurs particuliers sur lesquels je ne savais rien du tout auraient fort bien pu être la cause première de ces long sommeils. C'est la seule façon d'expliquer certaines réactions apparemment contradictoires obtenues en 1938–1939.

Par ailleurs l'hétérogénéité des échantillons constituant mes lots a quelquefois invalidé mes résultats et, à moins qu'elle ne fût très significative, il n'était pas toujours possible d'attribuer aux traitements la différence de développement observée entre deux lots. En effet, il y avait d'autres causes de variations, régionales, alimentaires, ou peut-être même génétiques qui n'avaient pas été éliminées et qui pouvaient avoir sur les sujets une plus grande influence que les traitements expérimentés. Il m'est arrivé par exemple d'avoir des pourcentages d'émergence très différents pour des lots placés dans des conditions presque identiques. Sur examen des échantillons formant ces lots, j'ai constaté que plusieurs individus, de volume inférieur à la moyenne, fai-

saient partie d'échantillons contenant un nombre considérable de larves élevées dans des bocaux trop petits, d'où insuffisance de nourriture. Ces larves étaient évidemment moins résistantes que les larves normales du deuxième lot, de là un plus fort pourcentage de mortalité et moins d'émergence.

En 1940, j'ai réduit les causes incontrôlables de variations en me procurant un stock aussi homogène que possible. Tous les cocons de *D. polytomum* (50,000) furent recueillis à la même date, le 26 août et au même endroit, dans un peuplement mêlé (épinette, sapin, et bouleau), à Ste-Florence, comté de Matapédia. Quant aux 25,000 cocons de *P. erichsoni*, ils furent ramassés entre le 26 août et le 1er septembre dans le canton Thorne, comté de Pontiac.

Espèces étudiées

Diprion polytomum Htg.

Pour une meilleure compréhension des expériences qui vont suivre il me paraît indispensable de dire quelques mots sur le cycle de *D. polytomum*. Auparavant, je dois d'abord attirer l'attention sur la classification de Benson (14) qui inclut *D. polytomum* dans son nouveau genre *Gilpinia* séparé de *Diprion* surtout par le développement des "cenchri" du meso- et metascutellum. Les caractères invoqués par Benson justifient peut-être l'érection de ce nouveau genre, mais comme l'usage de ce nom est encore plutôt restreint, j'ai cru préférable d'utiliser ici *Diprion polytomum*, nom sous lequel l'espèce est maintenant connue de tous les forestiers de Québec.

Cette Tenthrède apparemment introduite d'Europe et trouvée dans la Gaspésie à l'état épidémique en 1930 (8) ne s'attaque qu'à l'épinette. L'insecte hiverne dans la mousse ou l'humus à l'état larvaire dans un cocon brun. Au début de l'été, un pourcentage plus ou moins grand des individus se transforment d'abord en pronymphe puis en nymphe et finalement en adulte dont la plupart sont des femelles. En effet, les mâles, sont très rares et la reproduction est parthénogénésique. L'adulte est plutôt indolent et bien qu'il puisse voler sur de longues distances, il ne prendra son vol que par des journées ensoleillées, lorsque la température est relativement élevée. Les oeufs sont pondus à raison de un par feuille sur le vieux feuillage. L'éclosion de la larve se produit de 8 à 12 jours plus tard; elle se nourrit de préférence sur le vieux feuillage ne s'attaquant à la nouvelle pousse que plus tard dans la saison, et seulement lorsqu'il ne lui reste rien d'autre à sa portée. Au cours de sa vie larvaire l'insecte mue cinq fois et passe par six stages, alors qu'ayant atteint pleine maturité, il se laisse choir de l'arbre pour tisser son cocon dans la mousse. Entre le sixième stage larvaire, qui ne dure que quelques jours, et la pronymphe, certains auteurs reconnaissent un autre stage, celui d'éonymphe, forme sous laquelle l'insecte hivernerait. Dans ce travail, l'éonymphe est tout simplement désignée par le mot larve. L'emploi de ce terme ne peut prêter à confusion puisque cette étude ne porte que sur le développement à l'intérieur du cocon comprenant trois stages, à savoir: la larve ou éonymphe, la pronymphe et la nymphe.

Dans le nord de la province il n'y a qu'une génération par année alors que dans le district de Montréal et des Cantons de l'Est on peut avoir deux générations et trois dans l'est des Etats-Unis.

Dans la Gaspésie des adultes sont éclos de cocons en diapause depuis six ans, la durée de cette léthargie variant suivant les régions et les années. D'après Balch (7) le pourcentage moyen d'émergence par année approcherait 20% dans la Gaspésie alors que dans le centre du Nouveau-Brunswick il serait d'environ 70%. Pour une même localité, ce pourcentage varie beaucoup d'une année à l'autre; dans le comté de Kamouraska seulement 2% des larves hibernantes ont donné naissance à des adultes à l'été 1934. Au même endroit, pour les trois années qui suivirent, le pourcentage d'émergence a varié de 12 à 28%. Suivant Balch, la température et l'humidité seraient des facteurs de première importance pour la rupture de la diapause. Un séjour d'environ trois mois à des températures au-dessous du seuil de développement serait nécessaire pour obtenir le maximum d'émergence. Ce maximum serait de plus influencé par le degré d'humidité et le contact de l'eau. Sous élevages contrôlés, Balch a obtenu pour 21 générations consécutives une lignée sans diapause. D'un autre côté, les descendants de femelles provenant de la Gaspésie montrent une forte tendance pour l'hibernation et, à l'exception d'un cas, il a été impossible de l'éliminer après la première ou la deuxième génération. Tout en admettant l'importance de la température et de l'humidité, Balch croit donc que chez *D. polytomum* la diapause est héréditaire et les variations chez différentes lignées ou races seraient de nature génétique.

Pristiphora erichsoni Htg.

Les insectes hivernant dans le sol ont généralement surabondance d'humidité. L'habitat du cocon de la mouche à scie de l'épinette, bien que souvent saturé, est, cependant, relativement sec, surtout lorsque la larve s'est développée sur l'épinette blanche dans des peuplements mêlés où le sol est généralement bien drainé. Par contre, le cocon de la mouche à scie du mélèze étant dans un milieu presque toujours saturé d'eau et souvent même en contact avec l'eau, cette espèce est bien représentative des Tenthrèdes hibernant en milieu excessivement humide. Soumis à l'action des mêmes agents, cet insecte devrait donc dans maints cas réagir différemment de *D. polytomum*. C'est pourquoi, en 1940, la plupart des expériences faites sur *Diprion*, furent répétées avec *Pristiphora erichsoni*.

Probablement indigène au pays, la mouche à scie du mélèze fut constatée en Amérique pour la première fois dans l'Etat du Massachusetts en 1881 par Sargent (cf. 52). Depuis, la mouche à scie du mélèze s'est révélée l'insecte le plus nuisible du mélèze. Son cycle vital ne diffère pas sensiblement de celui de *Diprion*. La larve hiverne aussi dans son cocon sous la mousse. Ici encore les mâles sont peu fréquents et la reproduction est partiellement parthénogénésique. Les oeufs sont pondus en rangées dans des fentes taillées sur les pousses terminales et les larves se nourrissent exclusivement des feuilles du mélèze.

Il n'y a dans la littérature que très peu de détails sur la durée de la diapause de la mouche à scie du mélèze. Parmi les nombreuses publications consultées, je n'en ai trouvé qu'une seule (5) où il en soit fait mention. Lors de ses études sur le contrôle naturel de *Pristiphora erichsoni* dans le Nouveau-Brunswick, cet auteur remarqua qu'en 1921 environ 25% des larves étaient encore dans leur cocon après un an d'hibernation. Depuis, plusieurs années de manipulation de cocons de cette espèce pour la propagation de parasites ont permis à M. Baird (lettre du 12 novembre 1940) de constater que d'une année à l'autre et pour une même localité, il y a des variations considérables dans la durée de l'hibernation. Généralement, cependant, un très petit pourcentage des larves, moins de 1%, demeurent en léthargie pour plus d'un hiver, bien qu'il soit arrivé que ce pourcentage atteigne 25% et que quelques larves fussent encore vivantes dans leur cocon après deux ans.

Les observations faites par J. J. DeGryse (lettre du 24 octobre 1940) à Indian Head de 1923 à 1925, bien que de nature très générale, indiqueraient que dans l'Ouest du Canada la diapause peut durer deux et même trois ans. Des diapauses prolongées furent aussi observées il y a déjà plusieurs années dans l'Itasca Park, Minnesota (S. Graham, lettre du 21 avril 1941).

D'après mes propres expériences 1940–1941, il n'y a pas de doute que certaines années, dans certaines localités, la plus grande partie des larves subissent des diapauses qu'il est très difficile de rompre. Il est vrai que les traitements que j'ai fait subir aux individus de cette espèce causèrent dans plusieurs cas 100% de mortalité. Il n'en reste pas moins, cependant, qu'à la fin de mes expériences j'avais encore plus d'un millier de sujets vivants et sur ce nombre j'en ai eu moins d'une dizaine d'adultes. Pourtant les mêmes traitements en 1938–1939 avaient provoqué jusqu'à 40% d'émergence. Je ne puis expliquer cette différence de comportement qu'en l'attribuant aux conditions de milieu dans lesquelles vécurent ces individus avant leur arrivée au laboratoire, conditions ayant provoqué des diapauses tellement durables qu'il me fut, dans la majorité des cas, impossible de les briser au moyen des traitements employés.

Autres Espèces

Alors que les études de 1940 portèrent uniquement sur *Diprion polytomum* et *Pristiphora erichsoni*, quelques observations supplémentaires furent faites en 1938–1939 sur les Tenthrèdes suivantes en plus, bien entendu, des deux espèces ci-dessus mentionnées qui ont toujours constitué la plus grande partie du matériel.

Espèces	Nombre de spécimens
Pikonema alaskensis	662
Pikonema dimmockii	234
Neodiprion lecontei	319
Neodiprion pinetum	108
Hylotoma pectoralis	273

Technique

Saison 1938–1939

A l'automne de 1938, nous avions un total de 13,500 cocons obtenus grâce à notre enquête sur les insectes forestiers. Tous ces cocons furent répartis en 24 lots comprenant chacun 350 à 600 individus. Nous avons pris soin d'inclure dans chaque lot des échantillons de 1 à 10 cocons provenant de diverses parties de la Province et gardés séparément dans des fioles de 15 × 45 mm. bouchées avec de l'ouate. Le 1er octobre tout le matériel fut expédié dans un entrepôt frigorifique et remisé pour des périodes variant de 4 à 14 semaines dans des chambres à températures constantes soit: 32° F., 15° F., et 0° F., respectivement. Les abaissements de température furent graduels, c'est-à-dire qu'avant leur séjour à 0° F., par exemple, les individus passèrent d'abord trois jours à 45° F. puis trois jours à 32° F. et à 15° F.

De l'entrepôt frigorifique, les cocons furent expédiés au Collège Macdonald et amenés progressivement, par bonds de 15° F. (trois jours d'exposition à chaque échelon), aux températures de 60° F., 75° F., 80° F., 85° F., et 90° F., dans une atmosphère très sèche soit: 20 à 22% d'humidité relative dans les chambres de 85° F. et 90° F., environ 25% pour celles de 75° F. et 80° F., et 35% dans la chambre de 60° F. Il est à remarquer qu'au cours du trajet de Québec à Ste-Anne de Bellevue (une journée) il me fut impossible de contrôler la température. On m'a assuré, cependant, qu'elle oscillait entre 34° F. et 45° F.

A leur arrivée au Macdonald, chaque lot fut subdivisé en deux ou trois sous-lots de 150 à 200 cocons et soumis aux températures de nymphose ci-haut mentionnées. Auparavant, cependant, un échantillon de 50 cocons par lot fut prélevé et analysé afin de déterminer le taux de mortalité au cours de l'hibernation, ce taux étant ensuite appliqué aux sous-lots constituant le lot original.

Finalement, après un séjour de deux mois aux températures de nymphose, tous les cocons furent ouverts, et le taux de mortalité et de développement en incubateur fut calculé. Les chiffres obtenus furent alors utilisés pour estimer l'action de différents milieux sur les indivus.

Saison 1940–1941

La technique employée et les appareils utilisés en 1938 furent grandement améliorés en 1940. Tout d'abord nous avons cherché à obtenir un stock de cocons aussi homogène que possible en prélevant nos échantillons au même endroit et vers la même date. Cette façon de procéder a réduit au minimum les variations dues à l'origine des individus. A leur arrivée à Québec, les cocons de *D. polytomum* furent répartis en lots de 125 cocons et ceux de *P. erichsoni* en lots de 110 cocons, placés dans des tubes de verre de 22 × 95 mm., dans lesquels l'aération fut assurée en bouchant les extrémités avec de la mousseline.

Dès le 1er septembre, tous les cocons furent placés à basses températures dans notre réfrigérateur. Cet appareil comprend trois chambres tout à

fait indépendantes et qui peuvent être réglées à n'importe quelle température entre 0° F. et 45° F. au moyen de thermostats De Khotinsky. Dans nos expériences nous avons utilisé trois températures, soit 15° F., 34° F., et 45° F., mais la plupart des individus hibernèrent à 34° F. A la fin de l'hibernation, le taux de mortalité fut calculé en prélevant 25 cocons de chaque lot de *D. polytomum* et 10 de chaque lot de *P. erichsoni*. En conséquence il restait exactement 100 individus par lot lorsque les cocons furent transférés à l'incubateur. Le nombre d'individus examinés dans chaque lot afin de déterminer le taux de mortalité durant l'hibernation peut paraître plutôt faible, mais il est au contraire suffisamment élevé parce qu'au cours de l'hibernation, de 4 à 10 lots reçurent le même traitement.

Les expositions aux hautes températures eurent lieu dans trois incubateurs de 6 × 3 × 3 pieds à double parois de "masonite". La chaleur étant fournie par une plaque chauffante, contrôlée par un thermostat De Khotinsky, il n'y avait donc dans les incubateurs aucune source lumineuse. L'humidité désirée fut maintenu au moyen de l'appareil décrit par Fulton (43) et contrôlée par un humidistat "Fries". Incidemment, cet appareil de Fulton est peu dispendieux et il fonctionne très bien dans nos incubateurs. Pour la ventilation nous employons un éventail, à mouvement oscillatoire, dont la vitesse est réglée par un rhéostat. Afin d'assurer une bonne ventilation dans chaque lot avec le moins de perte d'espace possible, les incubateurs, de même que le réfrigérateur, sont équipés de chevalets de 1.5 × 2 × 2 pieds en tôle galvanisée avec sur les côtés des encoches à tous les trois pouces, sur lesquelles embarquent les treillis métalliques supportant les tubes de verre contenant les cocons. Les incubateurs furent réglés de façon à obtenir les températures et les humidités suivantes: Incubateur *A*, 75° F., 56% R.H.*; Incubateur *B*, 85° F., 85% R.H.; Incubateur *C*, 75° F., 85% R.H. D'après nos records de thermographes et hygrographes, les premiers vérifiés trois fois par jour sur des thermomètres Taylor, nous avons pu contrôler la température à 1° F. près et l'humidité relative à 3%.

Comme en 1938, toutes les expériences furent discontinuées après 60 jours d'incubation. Les cocons furent alors ouverts et les individus classés dans une des quatre catégories suivantes: parasités, morts, éclos, et vivants; les individus morts et vivants étaient de plus subdivisés en: larves, pupes, et adultes. Finalement les pourcentages de mortalité et d'émergence furent calculés comme suit:

$$\text{Pourcentage de mortalité} = \frac{100\ (\text{nombre de cocons morts})}{(\text{nombre total de cocons}) - (\text{cocons parasités})}$$

$$\text{Pourcentage d'émergence} = \frac{100\ (\text{éclos} + \text{pupes et adultes vivants après 60 jours d'incubation})}{(\text{nombre total de cocons}) - (\text{cocons parasités et morts})}$$

Les mots développement et émergence tels qu'employés dans ce travail ne sont pas tout à fait appropriés. En effet pour calculer le pourcentage de développement réel, si l'on considère la rupture de la diapause seulement, il

* R.H. = *Humidité relative.*

aurait peut-être été préférable d'ajouter au numérateur de la formule les pupes et adultes morts. Mais ce qui m'intéressait tout d'abord, ce n'était pas tant de démontrer l'influence de tel ou tel facteur sur la rupture de la diapause, comme son influence sur le pourcentage d'éclosion, car au point de vue économique c'est ce qui compte le plus. En outre le mot émergence n'est pas non plus très exact pour désigner le pourcentage d'émergence tel que calculé, puisqu'avec les cocons éclos sont inclus ceux contenant des pupes et adultes vivants. Il est fort probable, en effet, que si les expériences avaient été prolongées de quelques jours, l'éclosion aurait eu lieu.

Enfin, pour le calcul du pourcentage d'émergence, non seulement les cocons parasités, mais aussi les cocons morts furent éliminés, car dans la plupart des cas la majorité de ces individus étaient morts avant le commencement de l'expérience. Il est arrivé que les traitements subis par certains lots ont occasionné une mortalité plus élevée que la moyenne, mais ces cas furent mis à jour par les analyses de variance et furent pris en considération lors de la comparaison des différents traitements.

Influence de la température et de l'état hygrométrique de l'atmosphère

Action des températures d'hibernation

En 1938–1939, trois températures d'hibernation furent mises à l'essai: 32° F., 15° F., et 0° F. En 1940–1941, bien que tous les lots de *Pristiphora erichsoni*, ainsi que la plupart de ceux de *Diprion polytomum* hibernèrent à 34° F., plusieurs expériences furent effectuées à 45° F. et 15° F.

L'effet de la température d'hibernation a été considéré sous quatre aspects: 1. Mortalité durant l'hibernation; 2. Limite vitale inférieure; 3. Mortalité durant la nymphose; 4. Action sur l'émergence.

Mortalité durant l'hibernation

Comme le font soupçonner les résultats présentés au Tableau I et comme le prouve d'ailleurs l'analyse statistique des mêmes résultats (Tableau II), la valeur de "F" obtenue pour les variations dues aux traitements 1.42 nous donne une probabilité supérieure à 5% parce que plus petite que la valeur de *F* qui, avec une probabilité de 5%, est de 1.97 lorsqu'on a 11 et 55 degrés

TABLEAU I

Diprion polytomum, pourcentage de mortalité durant l'hibernation

Température d'hibernation	Durée de l'hibernation en jours			
	42	56	84	112
	Mortalité			
45° F.	20.0	25.3	21.3	20.0
34° F.	17.3	20.7	28.7	19.3
15° F.	17.3	28.7	20.7	22.0

TABLEAU II

ANALYSE DE VARIANCE SUR LA MORTALITÉ DURANT L'HIBERNATION

(Voir Tableau I)

Sources de variation	Degrés libres (*D.L.*)	Variance
Répétitions	5	157.16*
Traitements	11	88.40
Erreur	55	62.22
Total	71	Erreur standard = 7.89

* *Dépasse "erreur" variance, niveau significatif de 5%.*

libres (108). Si l'on considère, comme je l'ai fait partout dans mes analyses, que les traitements ou facteurs étudiés sont responsables des différences observées seulement lorsque celles-ci ont une probabilité inférieure à 5%, alors les différences constatées dans la moyenne de mortalité aux trois températures d'hibernation éprouvées en 1940 seraient simplement l'effet de fluctuations dues à l'échantillonnage. De plus la mortalité n'est pas influencée par la durée du séjour à 45° F., 34° F., ou 15° F., que ce séjour soit de 42 ou de 116 jours.

Le taux moyen de mortalité enregistré à la fin de l'hibernation pour les 72 lots exposés aux températures ci-haut mentionnées, fut de 22.8%. Ceci ne veut pas dire, toutefois, qu'un tel pourcentage d'individus aient succombé au cours de l'hibernation, car plusieurs étaient déjà morts au début des expériences et toutes ces moyennes ne peuvent donc servir qu'à comparer différents traitements et à estimer la mortalité en incubateur.

Limite vitale inférieure

Aucune des espèces étudiées en 1938 n'a pu résister très longtemps à une température de 0° F. Dans mes expériences, en effet, 38% des larves de *D. polytomum* étaient mortes après un stage de sept semaines à 0° F. Une semaine plus tard le taux de mortalité atteignit 65% et, après 10 semaines à 0° F., il ne restait plus qu'une seule larve vivante sur un total de 190 cocons. Dans ce dernier cas, environ 50% des individus avaient conservé l'apparence de larves vivantes, mais leur corps était flasque et si on les pressait légèrement elles ne reprenaient pas leur forme normale comme c'est le cas pour les larves en diapause seulement. Le reste des sujets avaient déjà pris une teinte jaunâtre et la tête était d'un vert foncé.

Dans un lot contenant 50 cocons de *Pristiphora erichsoni* et 45 d'*Hylotoma pectoralis*, il ne restait plus une seule larve vivante après un stage de 10 semaines à 0° F. Après 14 semaines à la même température, l'examen d'un lot constitué de 170 cocons de *Neodiprion pinetum* décelait 100% de mortalité.

Chez les Tenthrèdes, la limite vitale inférieure, lorsque les larves sont en diapause dans le cocon, nous paraît être entre 15° F. et 0° F.

Mortalité durant la nymphose

A la fin de nos expériences après 60 jours en incubateur, la moyenne de mortalité aux trois températures d'hibernation fut, comme suit (Tableau III): à 45° F., 43.5%; à 34° F., 47%; à 15° F., 47.9%. Comme on le voit, les températures d'hibernation par elles-mêmes n'affectent en rien le pourcentage de mortalité subséquente en incubateur.

TABLEAU III

Diprion polytomum, INFLUENCE DE LA TEMPÉRATURE ET DE L'HUMIDITÉ SUR LE POURCENTAGE DE MORTALITÉ EN INCUBATEUR

Hibernation		Temp.	R.H.	Temp.	R.H.	Temp.	R.H.
Température	Durée en jours	85°	85%	75°	85%	75°	56%
		Mortalité					
45° F.	42	48.9		40.0		55.6	
	56	37.9		47.9		28.8	
	84	47.8		34.0		45.7	
	112	35.2		43.1		47.1	
34° F.	42	66.5		27.0		67.3	
	56	25.0		32.2		36.9	
	84	47.3		49.9		68.9	
	112	45.1		45.6		70.8	
15° F.	42	36.3		35.8		83.9	
	56	26.0		39.6		48.3	
	84	37.5		51.4		40.7	
	112	60.7		52.7		61.4	

Par ailleurs, la résistance de *Diprion polytomum* aux différentes températures et humidités de nymphose est influencée par le degré de froid où s'est effectuée l'hibernation. En effet, au Tableau IV, la variance de l'interaction de la température d'hibernation sur les conditions de nymphose est 5.28 fois plus grande que la variance de l'erreur expérimentale, laquelle est associée aux variations dues au hasard et désignée par plusieurs, simplement par le mot "erreur". L'erreur standard de la moyenne pour cette interaction est égale à:

$$\frac{E.S.}{\sqrt{N}} = \frac{9.20}{\sqrt{8}} = 3.253$$

L'erreur standard de la différence entre deux moyennes étant $3.253\sqrt{2} = 4.60$, et la valeur de "t" pour $p = 5\%$ avec $n = 35$, étant 2.03, alors pour que la différence entre deux moyennes de mortalité à différentes températures d'hibernation et dans différents milieux de nymphose soit bien significative elle devra donc être supérieure à $4.60 \times 2.03 = 9.34$.

A 85° F., et 85% R.H., le pourcentage de mortalité n'est pas affecté par la température d'hibernation: la moyenne minimum étant de 40.2% à 15° F.

TABLEAU IV

ANALYSE DE VARIANCE SUR LA MORTALITÉ DURANT LA NYMPHOSE

(Voir Tableau III)

Sources de variation	*D.L.*	Variance
Blocs	1	0.1
Traitements	35	398.35**
Temp.	2	127.15
Jours	3	953.50**
Nymphose	2	1391.68**
Temp. × jours	6	165.54
Temp. × nymp.	4	432.62**
Jours × nymp.	6	403.39**
Temp. × jours × nymp.	12	241.67**
Erreur	35	84.2
Total	71	Erreur standard = 9.20

** *Dépasse "erreur" variance, niveau significatif de 1%.*

et la moyenne maximum 46.2% lorsque l'hibernation a lieu à 34° F. Il n'en est pas de même à 75° F. et 85% R.H., alors que la moyenne de mortalité n'est que de 33.7% à 34° F. d'hibernation et 44.9% à 15° F. Comme question de fait, lorsque l'on considère seulement les facteurs température et humidité, l'hibernation à 34° F., et la nymphose à 75° F. et 85% R.H. sont la combinaison donnant la mortalité minimum et l'émergence maximum. Incidemment, c'est ce que faisait prévoir mes résultats en 1938 et c'est pourquoi en 1940 la plus grande partie des expériences furent accomplies dans ces conditions de milieu.

Lorsque la nymphose se fait à 75° F., 56% R.H., la mortalité est beaucoup plus élevée si l'hibernation s'effectue à des températures voisines ou inférieures au point de congélation que si elle se produit à des températures bien supérieures à ce point. Ainsi, la moyenne de mortalité à 34° F., et 15° F., s'éleva à 61 et 58.6% respectivement, alors qu'elle ne fut que 44.3% à 45° F., ce qui est nettement significatif. *Diprion polytomum* est donc beaucoup plus sensible aux basses températures d'hibernation dans un milieu sec que dans un milieu humide.

Action de la température d'hibernation sur l'émergence

Chez *Diprion polytomum*, il n'y a pas de différence marquée dans le pourcentage de développement après une hibernation à 45° F. et 34° F. A 45° F., la moyenne d'émergence pour 800 individus fut de 10.7%, et à 34° F., pour le même nombre de cocons, j'obtins 9.8% d'émergence; cette différence est tout à fait négligeable comme le démontre l'analyse de variance du Tableau VI.

En 1940–1941, il me fut absolument impossible de provoquer la rupture de la diapause au moyen des températures et des humidités relatives ordinaires de nymphose après une hibernation à 15° F. Cependant, au cours de la saison

TABLEAU V

Diprion polytomum, INFLUENCE DE LA TEMPÉRATURE ET DE L'HUMIDITÉ SUR LE POURCENTAGE D'ÉMERGENCE

Hibernation		Températures et humidités relatives durant la nymphose			
		Temp.	R.H.	Temp.	R.H.
Température	Durée en jours	75°	85%	75°	56%
		Émergence			
45° F.	84	12.3		14.4	
	112	9.8		6.4	
34° F.	84	10.6		7.4	
	112	9.6		11.3	

TABLEAU VI

ANALYSE DE VARIANCE SUR LE POURCENTAGE D'ÉMERGENCE

(Voir Tableau V)

Source de variation	*D.L.*	Variance
Blocs	1	10.8
Traitements	7	13.10
Erreur	7	16.84
Total	15	Erreur standard = 4.104

1938–1939, sur un total de 1200 individus, la moyenne générale de développement après hibernation à 15° F. atteignit 12.7% avec un maximum de 22.7% pour un lot de 200 cocons à 75° F., 22% R.H. Ces résultats apparemment contradictoires au cours des deux années sont assez difficiles à expliquer. Il se peut que ce soit dû aux conditions différentes dans lesquelles s'est effectué le développement larvaire.

DÉTERMINATION DE LA TEMPÉRATURE ET DE L'HUMIDITÉ OPTIMA DE NYMPHOSE

Au cours de mes études préliminaires en 1938, les températures de nymphose mises à l'essai varièrent de 60° F. à 90° F., associées à une atmosphère toujours très sèche soit: 35% R.H. à 60° F., et 20 à 25% pour les températures de 75° F. et au-dessus. En 1940–1941, deux températures de nymphose, 75° F. et 85° F., de même que deux humidités relatives, 85 et 56% furent expérimentées.

Développement à 60° F., dans un milieu très sec

A 60° F., il y a reprise de développement, mais il va sans dire que le pourcentage est bien inférieur à celui que l'on obtient à des températures plus

élevées. Après une moyenne de 98 à 112 jours d'hibernation à 32° F., j'eus en 1939, pour *Diprion polytomum*, 8.5% de développement et 6.1% d'émergence, Tableau VII. A cette basse température, le pourcentage d'individus mourant dans le cocon à l'état de pupe ou d'adulte n'est donc que d'environ 2.4%, alors qu'à 75° F. et au-dessus il atteint 25 à 30%. Néanmoins, il est probable que le manque d'humidité (35% dans le premier cas et seulement 22% dans le dernier) fut en partie responsable de cette différence dans la mortalité.

TABLEAU VII

INFLUENCE DE LA TEMPÉRATURE ET DE L'HUMIDITÉ SUR LE POURCENTAGE DE DÉVELOPPEMENT ET D'ÉMERGENCE

(saison 1938–1939)

Hibernation		Température et humidité de nymphose					
		60° F., 35% R.H.		75° F., 22% R.H.		80° F., 22% R.H.	
Température	Jours	Dével.	Emerg.	Dével.	Emerg.	Dével.	Emerg.
32° F.	84	2.5	2.0	10.5	0.5	21.0	9.5
	98	5.7	4.3	41.5	17.0	59.1	11.0
	112	8.7	6.0	31.9	12.8	71.1	31.2
Moyenne		5.6	4.1	28.0	10.1	50.4	17.2
15° F.	84	*		14.3	6.5	3.2	2.2
	98			9.5	4.1	8.5	4.3
	112			22.7	13.4	18.1	3.2
Moyenne			-	15.5	8.0	9.9	3.2

* *Aucune expérience à 65° F., 35% R.H. après une hibernation à 15° F.*

Chez toutes les Tenthrèdes étudiées, après un stage aux températures d'hibernation, il y a reprise de développement à des températures aussi basses que 60° F.

Mortalité et développement à 75° F. et 80° F., dans des conditions hygrométriques variables

L'analyse de variance du Tableau IV indique que chez *D. polytomum* la mortalité durant la nymphose fut grandement affectée par les conditions de température et d'humidité que nous avions en incubateur. La moyenne de mortalité pour chacun des trois traitements fut comme suit: à 85° F., 85% R.H., mortalité 43.8%; à 75° F., 85% R.H., mortalité 40%; à 75° F., 56% R.H., mortalité 54.7%. Pour être significative, la différence entre ces moyennes doit être égale à:

$$\frac{9.2}{\sqrt{24}} \times 1.414 \times 2.03 = 5.4$$

Comme on le voit, la différence de mortalité aux températures de 75° F. et 85° F., en milieu très humide est négligeable. De fait, l'humidité relative, plus que la chaleur, influence la mortalité: une température de 85° F., en

milieu sec est nettement fatale à *Diprion.* Toutefois, si l'atmosphère est humide, le pourcentage de mortalité sera moindre à 85° F. qu'à 75° F. (température optimum) si cette dernière est associée à une atmosphère trop sèche (56% R.H.).

De plus nous avons vu précédemment que l'influence des températures d'hibernation sur le taux de mortalité est en rapport étroit avec le pourcentage d'humidité durant la nymphose. A des températures d'hibernation supérieures au point de congélation, l'humidité en incubateur n'influence pas la mortalité. Mais, à environ 32° F. ou à des températures inférieures, le pourcentage de mortalité est beaucoup plus élevé en milieu sec.

A la lumière de ces expériences on peut donc conclure que le milieu optimum de nymphose pour *Diprion polytomum* est de 75° F. à 80° F., avec une humidité relative de 85% ou plus.

A 75° F., dans un milieu très sec, Tableau VII, le pourcentage d'émergence chez *Diprion polytomum* n'est pas influencé par les températures d'hibernation. Cependant, lorsque la température de nymphose devient plus élevée (80° F.), il semblerait que la rupture de la diapause soit plus difficile si l'hibernation a eu lieu à de basses températures (15° F.). En effet, alors que j'obtins 50.4% de développement et 17.2% d'émergence après une hibernation à 32° F., il n'y eut, lorsque l'hibernation se fit aux environs de 15° F., seulement 9.9% de développement et 3.2% d'émergence.

En 1939, six lots placés à 75° F. et 22% R.H. présentèrent 27.9% de développement. En 1941, huit lots à la même température mais à 85% R.H. montrèrent un développement de 13% seulement. Ces différences peuvent s'expliquer en grande partie par l'origine différente des individus et par des conditions dissemblables de milieu au cours des deux années, car normalement on devrait s'attendre que le développement soit moindre à 22% d'humidité relative. Faut-il conclure de tout cela que les variations hygrométriques n'ont pas d'influence sur la rupture de la diapause? Il semble que oui, tout au moins pour tous les cas où les écarts dans l'état hygrométrique ne sont pas trop considérables. La preuve de ceci, c'est qu'en 1941 j'avais deux incubateurs réglés à 75° F., dont un à 85% R.H. et l'autre à 56% R.H. Il est vrai que la différence dans les conditions hygrométriques fut loin d'être aussi grande que pour les cas relatés plus haut, mais les résultats, Tableaux V et VI, démontrent tout de même de façon très nette que cette différence de 30% d'humidité relative n'affecte en rien l'émergence, que l'hibernation ait eu lieu à 34° F. ou 45° F., et qu'elle ait duré 84 ou 112 jours.

D'autre part, si l'état hygrométrique de l'atmosphère joue un rôle peu important dans la rupture de la diapause, par contre une forte déficience en humidité influencera considérablement l'émergence des adultes. En effet, en 1939, à une température de 75° F., l'émergence ne fut que de 10.1% à 25% d'humidité relative, alors qu'en 1941 avec 85% R.H., elle atteignait 10.6%. Comme j'avais 27.9% de développement en 1939 et 13% en 1941, ceci veut donc dire que dans une atmosphère très sèche, 17.8% des individus moururent à l'état de pupe et surtout d'adulte dans leur cocon contre seule-

ment 3.2% à 85% R.H. Ceci s'explique par le fait qu'en milieu sec le cocon est beaucoup plus dur et souvent l'adulte ne peut réussir à couper sa capsule de sortie. Dans l'incubateur de 80° F., 22% R.H., le nombre d'adultes morts dans leur cocon fut encore plus considérable. Ainsi, sur un total de 96 cocons ouverts après 12 semaines à 34° F., suivi de 60 jours à 80° F., 22% R.H., il y avait 46 adultes morts; 28 de ces adultes avaient commencé à couper leur trou de sortie mais moururent avant d'avoir terminé leur travail. Le même état de chose s'est répété partout où, en 1939, le développement fut réellement significatif. C'est ce qui explique une si grande marge entre le pourcentage de développement et d'émergence au Tableau VII.

Influence de variations brusques de température

D'après certains auteurs, non seulement le froid, mais toute variation extrême des températures normales aiderait à provoquer la rupture de la diapause. Ces variations brusques ou chocs thermiques, comme les appelle Cousin (30), amèneraient la reprise du développement là où des changements graduels ne produisent que peu ou pas d'effet.

Afin de vérifier si de telles variations sont effectives chez *D. polytomum*, les traitements suivants furent donc essayés en 1940–1941.

C. Dès leur arrivée au laboratoire, après avoir subi dans leur milieu naturel des températures d'environ 45° F. à 50° F., divers lots de cocons furent placés pendant deux semaines dans l'incubateur réglé à 75° F. et 85% R.H. Ensuite, ils furent transférés à 34° F., pour des périodes variant de 6 à 16 semaines puis ramenés directement à 75° F., pour 60 jours.

E. A la fin de l'hibernation, variations brusques de 34° F., à la température d'incubateur, mais sans exposition à 75° F., au début de l'hibernation.

F. Quatre jours avant la fin de l'hibernation, traités comme suit: deux jours à 75° F., suivi de deux jours à 34° F., puis retournés directement à l'incubateur de 75° F., pour la période de nymphose (60 jours).

G. Au début de l'hibernation, deux jours à 34° F., suivi de deux jours à 75° F., puis retournés à 34° F., pour les périodes habituelles variant de 6 à 16 semaines. A la fin de l'hibernation, passage graduel à 75° F., 85% R.H.

Mortalité durant la nymphose

Le pourcentage de mortalité constaté pour les traitements mentionnés ci-haut est présenté au Tableau VIII et l'analyse statistique en est faite au Tableau IX.

Cette analyse prouve que le nombre de jours en hibernation influence le pourcentage de mortalité durant la nymphose, mais ceci n'a rien d'anormal et ne présente qu'un intérêt secondaire. Ce qui nous intéresse dans le moment, c'est de connaître l'influence des variations brusques de température et, comme on le voit par la valeur de F qui n'est que 1.15 comparée à 3.29

TABLEAU VIII

Diprion polytomum, INFLUENCE DE VARIATIONS BRUSQUES DE TEMPÉRATURE SUR LE POURCENTAGE DE MORTALITÉ DURANT LA NYMPHOSE

Hibernation en jours	Types de variations de température			
	C	*E*	*F*	*G*
	Mortalité			
42	17.9	35.9	17.5	41.3
56	39.0	34.2	37.5	54.4
84	49.5	30.7	53.7	38.9
112	68.5	46.1	53.8	40.7

TABLEAU IX

Diprion polytomum, ANALYSE DE VARIANCE SUR LA MORTALITÉ DURANT LA NYMPHOSE

(Voir Tableau VIII)

Source de variation	*D.L.*	Variance
Blocs	1	.21
Traitements	15	351.39**
Jours	3	784.13**
Variations	3	86.76
Jours × variations	9	295.36**
Erreur	15	75.13
Total	31	Erreur standard = 8.668

** *Dépasse "erreur" variance, niveau significatif de 1%.*

pour $P = 5\%$, aucune des différentes variations expérimentées n'a été par elle-même la cause d'une mortalité réellement plus élevée. Par ailleurs, dans le cas de variations brusques de la température d'hibernation à celle de nymphose, traitements *C*, *E*, *F*, le pourcentage de mortalité augmente avec la durée du séjour à 34° F.

En outre, si l'on compare ces pourcentages de mortalité, Tableau VIII, avec celui obtenu après une hibernation à 34° F. (39.98%) on constate que les variations brusques de température au début ou à la fin de l'hibernation ne réduisent pas la vitalité de *Diprion polytomum* lors de la période de nymphose.

Émergence

D'après les résultats présentés au Tableau X, il n'y a aucun doute qu'un stage prolongé à de hautes températures au début de l'hibernation, après que l'insecte est entré en diapause, rend beaucoup plus difficile la rupture de

la diapause lorsque l'insecte est ensuite placé dans un milieu facilitant normalement la nymphose. Par contre, aucun des traitements *E*, *F*, ou *G* ne provoque un pourcentage d'émergence supérieur aux autres.

TABLEAU X

Diprion polytomum, INFLUENCE DE VARIATIONS BRUSQUES DE TEMPÉRATURE SUR LE POURCENTAGE D'ÉMERGENCE

Hibernation en jours	Types de variations de température			
	C	*E*	*F*	*G*
	Émergence			
84	2.5	25.3	11.8	10.0
112	2.0	15.0	23.5	20.2

La comparaison du développement obtenu après un passage graduel des températures d'hibernation à celles de la nymphose, Tableau V, avec le développement après des variations brusques de température est intéressante. Pour être significative, la différence dans le pourcentage d'émergence entre les deux groupes de traitements comparés doit être au moins de:

$$2.36 \sqrt{\left(\frac{4.104}{\sqrt{8}}\right)^2 + \left(\frac{4.801}{\sqrt{4}}\right)^2} = 2.36 \times 2.8 = 6.63$$

Lorsque le changement de la température d'hibernation à celle de la nymphose fut graduel, nous avions 10.9% d'émergence, Tableau V, alors qu'avec les traitements *E*, *F*, *G*, il fut de 20.2, 17.7, et 15.1 respectivement. Les variations brusques de température à la fin de la diapause, traitements *E* et *F*, provoqueront donc un développement beaucoup plus grand que les changements graduels dans la température, mais les variations brusques au début de l'hibernation n'ont, comme on pouvait s'y attendre, aucune supériorité marquée sur les changements graduels.

NYMPHOSE SANS EXPOSITION AUX BASSES TEMPÉRATURES

Lors de leur arrivée au laboratoire 300 cocons furent placés dans l'incubateur à 75° F., 85% R.H., sans passer par les températures d'hibernation. A tous les 15 jours, 50 cocons furent ouverts et l'état des individus noté. Comme les résultats ne présentèrent rien de particulier, inutile de les donner ici en détail. Signalons toutefois, qu'aucun développement ne fut observé.

On peut donc conclure de ces quelques expériences, qu'une fois que la larve de *Diprion polytomum* est entrée en diapause, il faut nécessairement un stage aux basses températures d'hibernation pour déclencher à nouveau le développement.

(La Partie II paraîtra dans le numéro de décembre.)

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